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STUDIES IN FRUIT STORAGE

I. INFLUENCE OF THE STAGE OF MATURITY AND STORAGE TEMPERATURE ON RESPIRATORY DRIFTS DURING THE RIPENING OF TOMATO FRUITS

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From the Institute of Agricultural Research, Benares Hindu University, India

(With 6 Text-figures)

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INTRODUCTION

INVESTIGATIONS in connexion with the storage of fruits by various workers have shown that the storage life of fruits can be prolonged considerably by suitable combinations of the three factors, temperature, carbon dioxide and oxygen concentrations in the storage room. With certain exceptions, low temperatures, high carbon dioxide concentrations and low percentages of oxygen depress respiratory activity in living tissue and thus retard the rate of physiological shrinkage during the storage of fruits. On the basis of experience in various storage investigations, a method has been developed which involves the storage of fruits at comparatively low temperatures with fairly high concentrations of carbon dioxide in association with low percentages of oxygen: this is designated the "refrigerated gas-storage" of fruits.

In the tropics, however, humidity is a factor of as great importance as any of those above mentioned. Weight losses due to low relative humidity can be checked more or less effectively by a suitable combination of high humidity and low temperature. It may be noted that atmospheres of the same relative humidity will have lower evaporating powers the lower the temperature. Among the other factors conditioning

the duration of storage life of fruits are variety, manurial treatment, maturity at picking, and diseases during storage.

The outlook for long-term storage of tomato fruits is not encouraging, but by attention to variety, manurial treatment, stage of maturity at picking and the environmental conditions of the fruits in the storage room, it is possible to extend greatly the time of marketing and to facilitate the long-distance transport of tomato fruits.

In this preliminary report the influence of two factors only, namely, the stage of maturity at picking and the storage temperature, on storage life and loss of weight during the storage period will be considered.

Literature

According to Wright *et al.* (1931) the tomato fruit tends to be intolerant of a severe retardation in its respiratory process by temperatures near 0° C. and to break down rapidly upon removal to higher temperatures after only short exposures to low temperatures.

In the temperate regions, Barker (1927) and Wright *et al.* (1931) have advocated storage temperatures in the range 10–15° C. for field-grown tomatoes, though somewhat lower temperatures appear to be permissible for tomatoes grown in the tropics.

Seasonal factors during the growth of the fruit are important in determining the storage life of tomato fruits. For example, Barker (1927) found that temperatures below 15·5° C. were injurious to English hothouse tomatoes, whereas the investigations of Wardlaw & McGuire (1932) indicate that tropically grown tomatoes are stored best at about 5° C.

Kidd & West (1932) found that English summer-grown fruit resembled the tropical fruit in physiological behaviour during storage, whereas the fruits grown in autumn were found to be intolerant of temperatures below 15° C.

Walford (1938) has recently published data dealing with the influence of season and the stage of maturity at picking upon certain aspects of the physiology of the fruit held at 12·5° C. during storage. Season was found to have a marked influence on the physiology of the fruit. He concluded that, while ripening on the plant, fruits pass through the senescent rise of respiration irrespective of the time of year in which they are grown in the greenhouse. In the summer fruit the senescent rise is evident in isolated fruits kept at 12·5° C., and the fruits are characterized by a lack of durability normal to this fruit. But in the winter fruit, if picked at maturity and still green, departures from the conventional type are obtained. The respiration record reveals an extended period of remarkably

steady rate while ripening is in progress and for long afterwards, suggesting a metabolically stable condition quite different from that obtained in the fruit of the conventional type.

Measurement of respiration

The fruits (var. Abundance) for this investigation were obtained from a tomato plot fertilized with 300 lb./acre of ammonium sulphate, 114 lb./acre of double superphosphate and 70 lb./acre of potassium sulphate.

After being picked from the vines, the fruits were brought immediately to the laboratory, the calyces were removed, the injured portions waxed and the weight of each individual fruit recorded. The fruits were kept in glass respiration chambers and the rate of respiration estimated by the Pettenkofer tube method. The absorption periods ranged between 24 and 48 hr. The respiration records represented in this paper refer to single fruits. Such a procedure is essential in connexion with studies on ripening of fruits, as individual variations are often masked if fruits of different developmental stages are put in the same lot for experimentation.

Results

The growth curves (Figs. 1, 2) as obtained by plotting successive fresh as well as dry weights against time show a slow increase in the beginning, followed by a very rapid increase which in turn ends by a gradual decrease and final cessation of growth. In Figs. 1 and 2, *A* and *D* refer to second trusses, *B* and *E* to fourth, and *C* and *F* to sixth trusses of two completely manured tomato plants. Considering the dry weight as the criterion of growth, the highest weight is attained by the fruits of the sixth truss (counting from the base) followed by that of the second and fourth trusses.

Fig. 3 records the respiration intensities and percentages of dry matter in fruits picked at different stages of development from the basal trusses of six tomato plants. The percentage of dry matter is highest in the young fruits, then declines and thereafter remains fairly constant throughout maturity and ripening. Respiration intensity is also highest in the young fruits and declines with further growth of the fruits. During the colour change from orange to red which is characteristic of ripening in tomato there is again a rise in respiration. This peak value has been described by the English plant physiologists as the "climacteric".

The data plotted in Fig. 4 have been obtained by following respiratory drifts of individual fruits picked at various stages of ripening (on the

basis of colour) and placed in storage at fairly constant temperatures of 14·7, 9·8 and 5·7° C. with adequate aeration. A significant point brought out by Fig. 4 is that irrespective of the stage of ripening at picking and the temperature of storage, winter field-grown tomatoes pass the

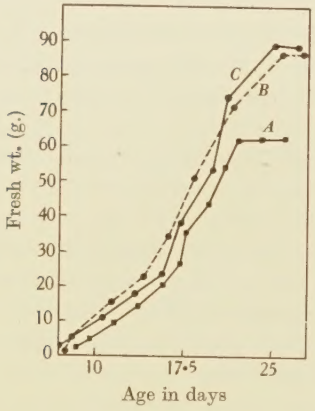

Fig. 1.

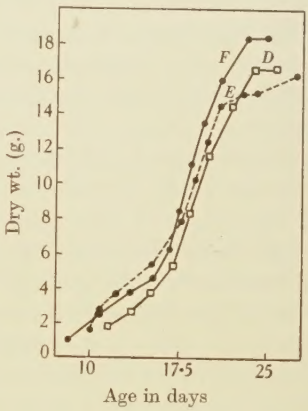

Fig. 2.

Figs. 1 and 2. Growth curves of tomato fruits constructed on the basis of fresh, as well as dry, weights. *A* and *D* refer to second trusses, *B* and *E* to fourth, and *C* and *F* to the sixth trusses of two completely manured tomato plants.

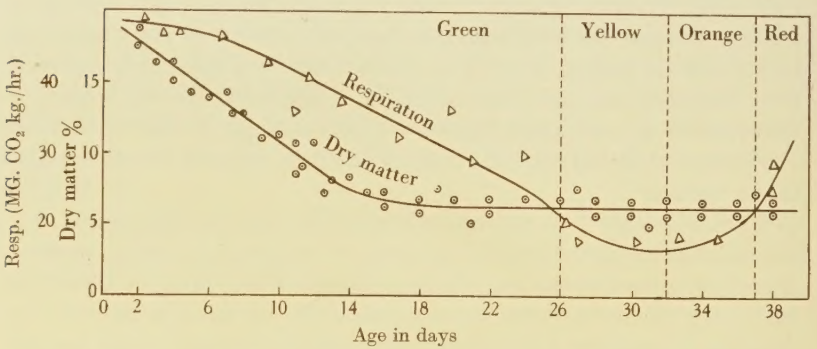

Fig. 3. Respiration intensities and percentage dry matter of fruits picked at different stages of development from the basal trusses of six completely manured tomato plants.

climacteric stage sooner or later. In Fig. 4 *green* refers to fully grown fruits having a green colour turning yellow, and *yellow* and *orange* refer to fruits picked in the yellow and orange stage respectively. A significant point observed was that certain tomatoes picked in the green stage showed

no evidence of the occurrence of the climacteric peak value during storage, though the usual sequence of colour change was recorded. One such case is shown graphically in Fig. 4 by bold dots. In this connexion it is interesting to note that Walford (1938) obtained different types of

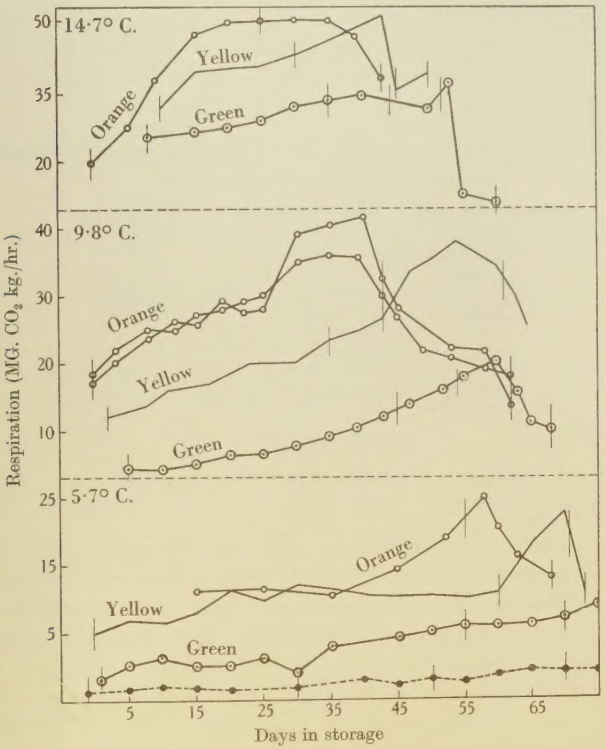

Fig. 4. Respiratory drifts during storage of green, yellow and orange fruits obtained from the basal trusses of four completely manured plants. The change of colour during storage is indicated by vertical lines crossing the curves, the order of the change in colour being green, yellow, orange, red. The colour at the commencement of the storage period is indicated on each curve.

respiration records with summer- and winter-grown tomatoes which he has designated as the "conventional" and the "anomalous" types. The autumn-winter-early-spring fruit, if picked approximately mature green, departs from the normal type. The respiration record reveals an extended

period of remarkably steady rate of metabolism and the storage life of the fruit is considerably increased.

Table I records the mean storage life in days and the mean physiological loss in weight during storage of fruits picked in the green, yellow and orange stages and placed in storage at 14·7, 9·8 and 5·7° C. The storage life of fruits is the longest at 5·7° C., followed by those stored at 9·8 and 14·7° C. At a given temperature the storage life is the longest in the case of green fruits and decreases progressively with increasing degrees of ripening of the tomatoes at the time of storage.

Table I. *Storage lives and physiological losses in weight of tomatoes stored at various temperatures*

Colour of the fruit at storage	Mean storage life days	Mean % loss in weight
	Storage temp. 14·7° C.	
Green	60	12·7
Yellow	50	12·1
Orange	43	12·0
	Storage temp. 9·8° C.	
Green	68	12·0
Yellow	65	11·8
Orange	62	11·6
	Storage temp. 5·7° C.	
Green	75	10·7
Yellow	73	10·7
Orange	68	10·2

When tomatoes are transferred from cold storage to higher temperatures for marketing, the excessive rate of respiration following this change of environment usually results in a more or less complete breakdown of the fruits. Experiments were, therefore, conducted to investigate the influence of a period of cold storage (20 days) at 4, 7, 10, 13 and 16° C. on the rate of respiration of green tomatoes on transference to 20° C. Relevant data obtained are shown graphically in Fig. 5. In general, when tomatoes are raised from lower to higher temperatures, the output of carbon dioxide at the higher temperature is temporarily increased above the level that it subsequently attains at that temperature. Fig. 5 further indicates that the lower the storage temperature the higher the initial respiration rate and the longer the time necessary for it to come to equilibrium at the higher temperature. After the attainment of the peak value at the higher temperature the rate of decrease is the greatest in the tomatoes stored at the lowest temperature, namely, 4° C.

More or less similar results were obtained with green tomatoes that had been in storage at 4° C. for 10 days and were transferred thereafter

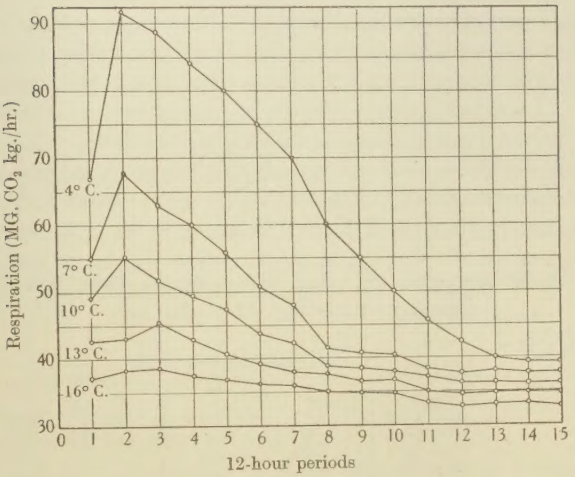

Fig. 5. Respiration rates in tomatoes from different storage temperatures (indicated on the curves). The respiration measurements were made at 20° C.

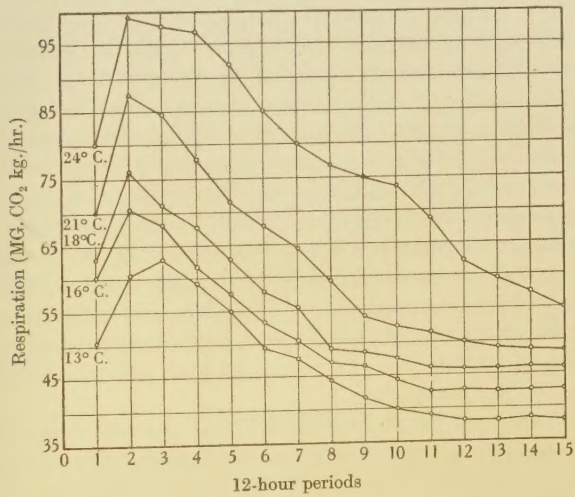

Fig. 6. Respiration rates in tomatoes from 4° C. storage. The temperatures given on the curves are those at which respiration determinations were made.

to 13, 16, 18, 21 and 24° C. for respiration determinations (Fig. 6). The initial high rate of respiration varies with the temperature at which the respiration determinations are made. The peak value of respiration intensity was obtained in 24 hr. in fruits whose respiration was measured at 16, 18, 21 and 24° C., whereas in tomatoes kept at 13° C. for respiration determinations the peak value occurred after 36 hr.

The values of R.Q. obtained on comparable lots of tomatoes by means of a gas-analysis method (Singh & Mathur, 1936 *b*) in both the series of experiments are presented in Table II. Generally speaking, the value of

Table II. *Values of* R.Q. *of tomatoes at successive* 12 *hr. periods after transference to higher temperatures*

Storage temp. (° C.)	Temp. (° C.) at which resp. was measured	12 hr. periods													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	13	1·16	1·17	1·14	1·11	1·11	1·09	1·08	1·07	1·09	1·06	0·99	1·00	0·99	1·00
4	16	—	1·14	1·12	1·11	—	1·09	1·08	1·09	1·08	1·09	0·99	1·01	1·01	1·02
4	18	1·19	1·12	1·12	1·17	1·17	1·11	1·11	—	—	—	1·00	0·99	0·99	1·02
4	21	1·17	1·13	1·13	1·27	1·20	1·17	1·11	1·09	1·07	1·08	1·02	1·00	0·98	1·01
4	24	1·21	1·29	1·31	1·30	1·21	1·05	1·04	1·03	0·99	1·07	1·08	1·00	0·99	0·99
4	20	1·22	1·23	1·27	1·20	1·20	1·19	1·17	1·16	1·17	1·16	1·09	1·01	1·00	1·00
7	20	1·20	1·20	1·26	—	—	—	—	1·17	1·09	1·08	1·07	1·01	1·00	1·00
10	20	1·19	1·17	1·22	1·19	1·17	1·11	1·15	—	1·06	—	—	0·99	1·01	1·01
13	20	1·19	1·20	1·20	1·16	1·16	1·12	1·14	—	1·05	1·05	1·05	0·98	1·02	1·03
16	20	1·18	1·19	1·19	1·10	1·11	1·12	1·17	—	1·05	1·05	1·05	1·01	1·03	1·00

R.Q. is considerably higher than unity immediately after transference to higher temperatures and drops down to approximately unity after 96–108 hr. A significant point observed in connexion with the values of R.Q. is that the R.Q. attains unity after 96–108 hr., but the rate of respiration is observed in certain cases, particularly in the fruits belonging to 4° C. storage, to be rapidly falling even after 144 hr.

The respiration curves obtained in fruits after transference from a lower to a higher temperature are therefore divisible in two parts: (i) high R.Q. associated with a rapidly falling respiration intensity, and (ii) a value of R.Q. equal to 1·0 accompanying either a falling respiration intensity or a level phase of the rate of carbon dioxide output depending upon the temperature of storage and the temperature at which the respiration determinations are made.

The first aspect of the problem, namely, the association of a high R.Q. with a rapidly falling respiration is probably attributable to (i) anaerobiosis, due to a rapid transference from low to high temperature, (ii) temporary flush at the higher temperature of carbon dioxide held in the sap and the atmosphere of the intercellular spaces during storage at

low temperatures, and (iii) accumulation of substances at low temperatures that give out carbon dioxide at higher temperatures without a proportionate intake of oxygen. In this connexion it may be noted that Singh & Mathur (1936*a*) have observed that considerable amounts of carbon dioxide accumulate in tomato fruits during ripening.

The second aspect of the problem, namely, values of R.Q. approximating to unity accompanying falling respiration intensity, is explicable on the basis of the assumption that respirable substances accumulate at low temperatures. In this connexion it is interesting to note that an accumulation of sucrose and reducing sugars usually occurs in potato tubers held at low temperatures (Hopkins, 1924; Barker, 1936).

Summary

1. The growth curves obtained by plotting successive fresh as well as dry weights are of the usual sigmoid type. The percentage of dry matter is the highest in young fruits and thereafter declines and remains fairly constant during maturity and ripening of fruits. The respiration curve during the growth of the fruits shows two high values separated in time: one is initial and represents a high rate of respiration in young fruits, while the other occurs at the onset of senescence.

2. Irrespective of the stage of ripening at picking, winter-grown tomatoes show the usual "climacteric" rise during the colour change from orange to red. In certain tomatoes there was no evidence of the occurrence of the climacteric, though the sequence of colour change from green through yellow and orange to red was of the usual type.

3. The storage life of the fruits is longest at 5·7 followed by 9·8 and 14·7° C. At a given temperature the storage life is longest in fruits picked green as compared to those picked in the yellow and orange stages.

4. The respiration curves obtained in fruits after transference from low to high temperatures are divisible into two parts: (i) high R.Q. associated with a rapidly falling respiration intensity, and (ii) a value of R.Q. approximating to unity accompanying either a falling or a steady respiration rate.

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A CANKER AND DIE-BACK OF ROSES CAUSED BY *GRIPHOSPHAERIA CORTICOLA*

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Botany School, Cambridge

(With Plates XIV and XV)

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Introduction

In January 1934 Dr G. E. Deacon of Brundall, Norwich, sent to the senior author some green stems of Kokulensky's *canina* rose stock bearing sunken brown lesions with a purplish margin (Pl. XIV, fig. 1). The lesions were $\frac{1}{2}$–$\frac{3}{4}$ in. long and extended about half-way round the stem; some were obviously the sites of rust attack (*Phragmidium mucronatum*), but others were not. On the dead bark there were numerous small, brownish black fructifications containing three-septate, light brown spores formed at the extremities of slender hyaline conidiophores; the basal cell of the spores was usually colourless. The fructifications were either acervuli or irregularly shaped pycnidia with an ill-defined ostiole. The fungus clearly belonged to the genus *Coryneum* or *Hendersonia*, several species of which have been recorded on rose stems. A culture of the fungus was established in order to test its pathogenicity. Further material of the disease was sought, and in addition to the first isolation the following cultures were established from roses:

No. 2: from a dead stem of Colchester *canina* rose stock sent by Dr Deacon, February 1934.

No. 3: from a dead stem of *Rosa Moyesii* collected by Dr d'Oliveira in the Cambridge Botanic Garden, March 1934.

No. 4: from a lesion on a green stem of an unknown cultivated variety sent by Dr Deacon, April 1934 (Pl. XIV, fig. 2).

No. 5: from a canker on a green stem of "Isobel" rose collected at Wisley, June 1934.

Nos. 6 and 7: from lesions on green stems of Kokulensky's *canina* rose stock collected near Norwich, December 1934.

No. 8: from a dead stem of an unknown cultivated variety, March 1935.

Nos. 9 and 10: from dead stems of unknown cultivated varieties sent by Dr Deacon, March 1935.

No. 11: from a canker on "Gloria Mundi" rose growing under glass at Keynsham, sent by Mr L. Ogilvie, Long Ashton, Bristol, March 1936.

In addition, the same disease was sent to us by Mr G. Samuel on "Karen Poulsen" roses from his garden at Harpenden and by Mrs M. Gregor from cultivated roses at Crieff, Scotland.

Symptoms of the disease

At the Field Station of the Cambridge Botany School, where there is a large collection of roses for experimental purposes, the disease was established by inoculation, after which it was allowed to spread spontaneously. The disease is confined to the stems and does not directly affect the leaves. It appears either as a canker or a die-back. The cankers begin to develop as brown depressions in the green bark, frequently around a dead bud, a thorn scar, a rust lesion or some other kind of injury. The margin of the canker is smooth and purplish in colour. The cankers usually extend with time to about half-way round the stem and they may be half an inch to several inches in length. As the bark dies numerous small blackish fructifications develop. After some time the canker usually ceases to spread; the bark becomes sunken, but the wood is not exposed and the margin does not become rough and swollen as it commonly does in the canker caused by *Coniothyrium Fuckelii* Sacc. In the young stages, however, the two kinds of cankers are hardly distinguishable unless the parasites are fruiting. Occasionally, the cankers caused by the disease now under consideration completely girdle the stem, when the upper part dies back. Alternatively, the fungus may infect a pruning wound and spread downwards extensively, again causing die-back. If cankers or stems killed by the fungus are allowed to remain on a susceptible variety of rose, the disease spreads somewhat rapidly on account of secondary infections from spores produced in the first lesions. Under these conditions "die-back" of the bushes becomes conspicuous.

Cultural studies

Single spore cultures were established from each of the isolations nos. 1–11 and were grown side by side on a variety of agar media such as Dox, potato dextrose, malt extract, oatmeal and rose stem extract, and also on sterilized stems of *Rosa canina*. The cultures spored best on Dox's medium and on rose stems, so the various isolations were maintained on these throughout the investigation. Subsequently, Miss A. E. Jenkins kindly sent two cultures (nos. 66 and 67) of *Coryneum microstictum* B. and Br. from roses in the United States, but only no. 66 spored freely.

On Dox's medium the cultures, including Miss Jenkins' no. 66, produced varying amounts of white aerial mycelium and dark spore aggregates which differed somewhat in abundance and colour in the respective isolations. The surface of the medium was sometimes practically covered with the spore masses, almost to the exclusion of aerial mycelium. Pl. XV, fig. 3, shows cultures of four of the isolations on Dox's agar. These spore aggregates arose directly from massed conidiophores: they were not formed in pycnidia nor was there a compact layer of hyphae below them as in a typical acervulus. There were minor differences in the shape, size and septation of the spores in the various isolations, which had been previously noted on the natural host. These differences will be referred to later.

On growing isolations nos. 1–10 on Dox's medium at different temperatures their growth rates were found to be essentially the same, the optimum temperature being about 20° C. Little growth occurred at 5 or 30° C., and isolation no. 4 did not grow at all at the latter temperature. Temperatures above 20° C. favoured the development of aerial mycelium to the detriment of spore formation.

On sterilized rose twigs the various isolations showed similar slight macroscopic differences. The conidial pustules on the surface of the twigs were built up in the same way as on Dox's medium. Typical pycnidia or acervuli were not formed in the bark. After about 3 months, however, isolations nos. 1, 5 and 6 gave rise to perithecia in the bark, the ascospores of which were three-septate and hyaline. The perithecia were first seen in culture in February 1935. The formation of perithecia enabled the fungus to be identified as *Griphosphaeria corticola* (Fckl.) v. Höhnel. Single ascospores were isolated and grown in culture: the same conidial stage was again produced. Soon after perithecia were seen in culture they were found on rose stems inoculated in the field with these

particular isolations. Perithecia have not been seen in connexion with the other conidial isolations, either in the field or in culture.

The conidia germinate readily in water and in nutritive media, each cell, except generally the hyaline basal one, forming a germ tube. Germination commences within a few hours at 20° C. The ascospores also germinate readily and each cell may form a germ tube somewhat stouter than that from a conidium. The ascospores occasionally germinate inside the ascus by the formation of germ tubes, when the ascus wall gradually breaks down. Germination of ascospores within the ascus to form sporidia, as observed sometimes by Petrak (1921), has not been seen.

It will be convenient here to describe the method of ascospore discharge from the perithecium. At maturity and under moist conditions the asci elongate successively so that the tips protrude slightly beyond the ostiole. An ascus, so extended, discharges its spores violently and then contracts, its place being taken by the next ascus ready to liberate its spores, and so on.

Inoculation experiments

A preliminary test of the pathogenicity of isolations nos. 1–5 was carried out during the summer of 1934 with the following varieties of rose bushes: Etoile de Hollande, Shot Silk, Lady Inchiquin, Golden Emblem, Independence Day, Duchess of Atholl, Mrs Sam McGredy, Mrs G. A. Van Rossen, Mabel Morse, Angele Pernet, Madame Edouard Herriot, Lady Pirrie, Ophelia, Madame Butterfly, Mrs Henry Bowles and Mrs A. R. Barraclough. T-shaped wounds were made in the bark of 2-year-old stems, and a culture of the fungus from Dox's medium was inserted under moist conditions. Within 6 months incipient cankers had been formed on several varieties by each of the isolations; on some of the cankers conidial fructifications had already developed on the dead bark, from which cultures were again established. As it was evident that all the isolations were pathogenic to roses many series of inoculations were subsequently carried out under more natural conditions and at different times of the year.

During November 1934 2-year-old stems of the above varieties were cut back and the exposed extremities inoculated with an aqueous suspension of conidia of isolations nos. 1–5, such wounds being similar to those made in pruning. It had already been ascertained that on applying a spore suspension to the cut extremity of a rose twig the spores were sucked a considerable distance into the wood vessels, as described by Brooks & Moore (1923) for *Stereum purpureum*. Some of the stems

inoculated in this way were left exposed to the air, while others were covered with cotton-wool which was kept moist for a week following inoculation. Adequate controls were maintained. In January 1935 it was evident that most of the inoculated stems were being successfully invaded, whether exposed or protected. Browning of the bark occurred from the cut end downwards, there frequently being a purplish zone at the junction of diseased and healthy tissues. At this date no change was detected in the controls. By May 1935 some of the inoculated stems had died back to the extent of 6–12 in. and nearly all were bearing conidial fructifications on the dead bark. A few of the inoculations were rendered worthless through secondary infection by *Botrytis cinerea*, as were also some of the control experiments. Apart from infection by *Botrytis*, however, the control stems had died back at the most only to the nearest bud. All the isolations nos. 1–5 again proved to be pathogenic, and to judge by the amount of die-back nos. 1 and 5 were the most lethal. In this experiment, as in several others, the varieties Golden Emblem, Duchess of Atholl and Lady Inchiquin were the most susceptible, and Independence Day, Mrs G. A. Van Rossen and Madame Edouard Herriot the most resistant, although no variety was entirely unaffected. In addition to conidial fructifications, perithecia of *Griphosphaeria corticola* were found in May 1935 on stems inoculated with isolations nos. 1 and 5. In connexion with these inoculations it was noticed that, in general, the disease ceased to progress during the summer following inoculation; once invasion had been stopped the disease did not subsequently extend.

A similar experiment carried out in November 1935 with isolations nos. 6, 7, 8 and 10, showed that these also were pathogenic, isolation no. 6 being the most active and resulting in the formation of perithecia of *Griphosphaeria corticola* as well as conidia.

It was then decided to ascertain whether infection of the cut ends of shoots was more likely to occur at some seasons of the year than at others, as described by Brooks & Moore (1926) for *Stereum purpureum*. Isolations nos. 1 and 5 were chosen for this series of experiments as being most pathogenic. Freshly cut stem extremities of the sixteen varieties of roses were inoculated with aqueous suspensions of conidia during January, March, May, July, September and November. Except for the inoculations made in July nearly all were successful, observations on the stems being continued until the end of the summer following inoculation. Most of the stems inoculated in July were not appreciably affected at all, but a few became invaded to the extent of about an inch. Several of these July inoculations were cut off and examined; sections showed that the

browning of the internal tissues associated with the progress of the fungus was intensified at the lower limit of invasion as if a "gum barrier" had prevented further spread of the disease. In the completely unsuccessful inoculations the "gum barrier" was situated just below the exposed end of the stem. Other evidence showed that the spores of the inoculum had germinated, but that advance of the mycelium was soon stopped by the reactions of the host tissues at this time of year. These experiments showed that it was virtually impossible for the fungus to cause infection during the middle of the summer when inoculation was carried out in a natural manner. With the November and January inoculations invasion was very slow until the rise in temperature in spring. Again, it was observed that further dying back of the stems ceased during the summer following inoculation, although considerable lengths of stem might have been killed before then.

Some evidence was obtained that if freshly cut ends of stems were left exposed for 3 months before inoculation it was difficult to bring about infection whatever the time of year. Unfortunately, however, the largest experiment designed to test liability to infection after varying periods of exposure was ruined by a severe attack of *Botrytis cinerea* which caused extensive die-back.

Another experiment was planned to determine whether the age of the tissues influenced invasion. Young, incompletely hardened stems and stems 1 and 2 years old were cut back and the exposures inoculated with a conidial suspension of isolation no. 1. Infection almost invariably occurred, and there was no indication that the age of the tissues appreciably influenced susceptibility. Natural infection, however, has not been seen in stems less than about 1 year old.

Infection through pruning wounds was obviously one channel of invasion, but in the naturally occurring specimens of the disease it had been noticed that infection sometimes centred round rust lesions, dead buds and thorn scars. Attempts were therefore made to ascertain whether infection could be induced experimentally through such injuries and also through leaf scars and wounds made by thorns. The varieties Golden Emblem, Duchess of Atholl, Mrs Sam McGredy, Lady Inchiquin and Etoile de Hollande were used for this purpose. As there were no rust lesions on the stems of the cultivated varieties stocks of *Rosa canina* affected by rust were used in this connexion. The dead buds, which were inoculated in April 1936, had been killed by frost shortly before then. Thorns were removed and the scars forthwith inoculated. Other wounds were deliberately made by scratching stems with the thorns of others,

thus simulating a type of injury which is common in Nature. All these kinds of injuries and some leaf scars were inoculated with an aqueous suspension of conidia of isolation no. 1, adequate controls being maintained. In due course characteristic cankers developed around the dead buds, rust lesions and wounds made by thorns, which had been inoculated with the fungus. Most of the inoculated thorn scars and a few of the leaf scars were similarly affected. The controls remained healthy. It is clear, therefore, that there are many ways in which this disease of roses can become established. With regard to infection through rust lesions it is of interest that Deacon (1938) has described infection by *Coniothyrium Fuckelii* through the same channel.

Certain kinds of rose stocks also proved to be susceptible to the disease. Only one series of experiments with stocks will be described, which concerned the following: *Rosa canina* (from cuttings), Kokulensky's *canina* (from seedlings), *R. multiflora polyantha* (from seedlings), *R. rugosa* (from cuttings), *R. rubiginosa* (from seedlings) and Manetti (from cuttings). Stems of these stocks were cut back in November 1935 and inoculated, as previously described, with conidial suspensions of isolations nos. 1 and 5. In the late spring of 1936 most of these inoculated stems had died back to the extent of about two inches and bore conidial pustules. *R. canina* and Kokulensky's *canina* were somewhat more susceptible than the others, but progress of the disease was not so rapid as in cultivated varieties of roses.

All the inoculations so far described were made with mycelium or conidia of the various isolations. Ascospores of *Griphosphaeria corticola* were also used for inoculation in May and November 1936. By teasing out the perithecia from the bark and suitable manipulation in water considerable numbers of ascospores were obtained free from admixture with conidia. Aqueous suspensions of ascospores were applied to freshly cut extremities of stems of the cultivated varieties or to T-shaped cuts made in the stems. All these inoculations were successful, resulting either in dying back of the stems or in the formation of cankers. Both conidial pustules and perithecia developed in the dead bark, thus providing further evidence of the relationship of the conidial stage to *G. corticola*.

Some inoculation experiments were carried out in October 1934 and October 1936 with Etoile de Hollande and Golden Emblem roses growing in pots in an unheated glasshouse, conidial suspensions of isolations nos. 1–5 being applied to freshly cut stem extremities. Infection resulted, but there was less die-back than with comparable inoculations in the

fields, doubtless owing to the earlier awakening of the host from dormancy in the glasshouse and consequent checking of the parasite. During the 1934 experiments the glasshouse atmosphere was dry and few conidial fructifications developed on the dead bark. During the experiments begun in October 1936 the atmosphere of the glasshouse was kept moist with the result that abundant conidial pustules developed. Ascospore inoculations were also successful in the glasshouse.

Pathological anatomy

The cankers caused by this parasite were studied anatomically at different stages of development. The mycelium spreads between the cortical cells, killing them as growth proceeds. The walls and contents of the cells become brown, and after death they may be penetrated by the hyphae. The mycelium may eventually reach the wood by way of the medullary rays, and brown gum is deposited in the cells. Occasionally the pith may be invaded, when it also becomes brown. Sometimes, however, the fungus enters the wood to no great depth, when a narrow darker zone of gummy material delimits the diseased from the healthy wood. In the cankered bark the mycelium spreads more rapidly in a longitudinal than in a lateral direction, and stabilized cankers are often seen which extend only partly round the stem. During summer the healthy bark on the margin of the canker forms a phellogen which produces a cork barrier that extends from the surface to the wood, thus preventing the extension of the canker during the current season. The xylem cells formed by the cambium on the border of the canker remain parenchymatous, and this region may also be traversed by a cork barrier continuous with that in the bark on the margin of the lesion. Opportunity for the lateral extension of the canker by invasion of the newly formed xylem is thus greatly reduced. In our experience further development of the canker after its progress has once been checked in this way is very rare. On the other hand, if the canker spreads all round the stem before it is delimited by a cork barrier, the stem above the canker is completely killed and the disease simulates a die-back.

It has been pointed out that where infection occurs through the cut extremities of stems, as in pruning wounds, extensive die-back may result, unassociated with the formation of cankers. A few words will first be said about the reactions of rose tissues exposed by pruning apart from invasion by a parasitic fungus. Study of these reactions shows that they are very similar to those in plum stems described by Brooks & Brenchley

(1931). Soon after wounding, the woody tissues become brown from the surface downwards owing to the formation of gum in the vessels and medullary rays, with the coincident disappearance of starch. This may be wholly a wound response or it may be associated with invasion by harmless micro-organisms. The browning of the tissues extends downwards in a V-shaped manner until a bud or lateral shoot is reached, at about which level a narrow darker zone of gum is formed. This has been termed a "gum barrier" as it appears to check parasitic invasion. The pith in a rose stem is wider than in a plum stem, and in the former a "gum barrier" is also formed at the appropriate level in the small living cells of this region. Corresponding with the "gum barrier" in the pith and wood a cork barrier is formed in the bark. Apart from parasitic invasion rose "snags" always die in this way. The time rate of these changes depends on the season of the year when the wound is made. If it is made in the winter the host reactions do not occur rapidly until spring, but if the tissues are exposed during spring or early summer the reactions follow speedily and the wound is soon rendered relatively innocuous.

When wounds such as those made in pruning are invaded by the parasite under consideration it grows downwards in the bark and wood, killing the tissues as it proceeds. The progress of the fungus in the wood is accompanied by the formation of gum. The hyphae pass from cell to cell through the pits, but exercise no disintegrating action on the membranes. During the summer, however, the growth of the fungus is usually stayed, after which invasion never appears to be resumed. On sectioning a stem in which invasion has stopped, a narrow, very dark zone of gum is seen at the junction of dead and healthy tissues (wood and pith), which the fungus seems unable to penetrate and which is therefore termed a "gum barrier", similar to that described by Brooks & Moore (1926) in plum stems where invasion by *Stereum purpureum* has been checked. At about the same level in the bark there is a cork barrier which also prevents further invasion. These barriers are usually formed in the vicinity of a lateral shoot. The extent of the die-back caused in this way depends on the time of year when infection begins and on the relative susceptibility of the variety. Evidence was obtained that "gum barriers" are formed more rapidly in resistant than in susceptible varieties of roses.

Identification of the parasite

The perithecial stage of the fungus has been identified as *Griphosphaeria corticola* (Fckl.) v. Höhnel. The genus *Griphosphaeria*, of which *G. corticola* is the type species, was established by von Höhnel (1918) on account of the peculiar character of the perithecial wall, most of which consists of hyphae that run parallel to one another and are closely pressed together. Petrak (1921) also has described *G. corticola* and has stressed the unusual structure of the perithecial wall. Both authors give long lists of synonyms, which include *Metasphaeria corticola* (Fckl.) Sacc. We have compared our fungus with Petrak's material of *Griphosphaeria corticola* on *Rosa canina* in the Kew Herbarium, and we are satisfied that it is the same species. Grove (1937) states that he and Mr E. A. Ellis (of Norwich) repeatedly found *Metasphaeria corticola* on twigs of wild roses in intimate association with *Coryneum microstictum* B. & Br. Mr Ellis has kindly allowed us to examine his specimens of *Metasphaeria corticola* on wild and cultivated roses, which agree exactly with our fungus. Dr G. E. Deacon reported to one of us in April 1935 that he had found perithecia (subsequently determined as *Griphosphaeria corticola*) on stems of rose stocks and cultivated varieties near Norwich. A survey of the literature shows that the fungus now known as *G. corticola* occurs commonly on dead stems of *Rosa*, *Rubus*, *Crataegus*, *Prunus* and other genera in Central Europe. As there is no English description of *Griphosphaeria corticola* the following diagnosis is given:

Perithecia scattered or aggregated, sunk in the substratum, brownish black, globose, 400–600μ in diameter, tapering upwards to the ostiole which becomes visible on the rupture of the overlying tissues; most of the wall consisting of two layers of closely compressed, parallel hyphae, the inner one hyaline and the outer one brown; asci cylindrical, thickened at the apex, 80–120 × 7–8μ, eight-spored; ascospores obliquely uniseriate, spindle-shaped, straight or somewhat one-sided, tapering towards the rounded ends, mostly three-septate, sometimes slightly constricted at the septa, 14–18 × 5–7μ, hyaline; paraphyses numerous, slender, longer than the asci, 2–3μ wide.

Illustrations of the perithecia are shown in Pl. XV, figs. 1, 2.

Greater difficulty has been experienced in naming the conidial stage. It has already been pointed out that some of the conidial isolations have given rise to perithecia whereas others have not. Another complication is that the conidial fructifications range from imperfectly formed

pycnidia (*Hendersonia*) to typical acervuli (*Coryneum*) even as regards the same isolation (Pl. XIV, figs. 3, 4). Such variation in development is in agreement with the conclusion of Archer (1926), concerning an allied fungus, who found that *Hendersonia Rubi* (West.) Sacc. is identical with *Coryneum ruborum* Oud. A further complication arises from the fact that the conidia of the various isolations, although all predominantly three-septate and brown (except for the usually hyaline basal cell), differ somewhat in shape, size and colour; these conidial isolations also show slight differences in cultural behaviour and in relative pathogenicity. Since, however, all these conidial isolations produce the same kind of disease in roses and since they are closely similar in structure, we incline provisionally to the view that they represent a complex of slightly differing types which in the present state of our knowledge should be referred to a single species. This view is fortified by the opinion of Grove (1937) who suggests that *Coryneum* (*Coryneopsis*) *microstictum* B. & Br., *Hendersonia canina* Brun., and *H. Henriquesiana* Sacc. & Roum. are all probably identical. Many other species of *Hendersonia* are recorded on roses in Saccardo's *Sylloge Fungorum*, and it is likely that most of these also are synonymous with *Coryneum microstictum*. Material of several of these species of *Hendersonia* and of *Coryneum microstictum* on roses preserved in the Herbaria of Kew and the British Museum (Natural History) has been examined and compared with our own specimens. Some of the specimens labelled as species of *Hendersonia* in these Herbaria are unquestionably identical with *Coryneum microstictum*. With regard to our own conidial collections, some agree exactly with *C. microstictum*, including those associated with perithecia of *Griphosphaeria corticola*; others diverge somewhat from it, but may nevertheless be included in it in view of the remarks made above. It seems best, therefore, to refer all the conidial isolations provisionally to *Coryneum microstictum*, some of which only have formed perithecia during these investigations. It is not unusual for certain strains of other Ascomycetes to lose the capacity of forming ascocarps. On the other hand, further research may show that not all the conidial isolations can be included in *C. microstictum*. Like *Griphosphaeria corticola*, *Coryneum microstictum* occurs on many other hosts as well as on roses. The following description of *C. microstictum* is given in the light of our own studies:

Acervuli or ill-defined pycnidia brownish black, sunk in the substratum and only partly exposed on the rupture of the host tissues; conidiophores slender, unbranched, 20–25 × 1·5 μ, hyaline; spores pear-shaped, ovoid or spindle-shaped, straight or rarely slightly curved,

mostly four-celled, light to dark brown except for the basal cell which is usually hyaline, 12–18 × 4–6·5μ.

Discussion

Hitherto only slight references have been made to this disease in accounts of the pathology of roses, although it is undoubtedly widely distributed. *Coryneum microstictum* and *Griphosphaeria corticola* have been frequently described from the morphological standpoint on dead stems of roses and other plants without reference to pathogenicity. Sorauer (1888) briefly described two *Hendersonia* diseases of roses which may perhaps be related to the disease described in this paper. Beauverie (1914) described a disease of roses in France which was associated with *Coryneum microstictum*. Averna-Saccá (1926) described a stem disease of cultivated roses in Brazil, which he attributed to *Hendersonia rosicola* n.sp. It appeared in the form of large spots or livid rings and sometimes caused wilting. Mr W. C. Moore (of the Plant Pathological Laboratory, Ministry of Agriculture) tells us that Miss Westcott and Miss Jenkins (from the United States) found a canker of roses at the Royal Horticultural Society's Garden at Wisley in 1930 which was associated both with a *Coryneum* or *Hendersonia* and perithecia of *Griphosphaeria corticola*. In *Bulletin* 79 of the Ministry of Agriculture (*Fungus and other Diseases of Crops*, 1928–1932) mention is made of the occurrence of a species of *Coryneum*, probably *C. microstictum*, on roses in Surrey in 1931 and in Monmouth in 1932. Jenkins (1937) has described a canker of roses in various parts of North America which is caused by *C. microstictum*.

This disease of roses, although fairly common in our experience, rarely occurs abundantly and is therefore liable to be overlooked. From our observations at the Field Station of the Cambridge Botany School, however, this canker and die-back can cause considerable damage to susceptible varieties of roses if allowed to spread without check. It occurs most frequently on certain varieties of hybrid tea roses, and as these are usually drastically pruned in the spring the lesions are generally cut off and destroyed. The destruction of the disease by fire is, in fact, the only course that can be recommended. With the Poulsen varieties of roses, which also are susceptible to the disease, cutting out of the affected parts will have to be done deliberately as these varieties are only pruned lightly if at all. The disease has also been reported on roses grown commercially under glass, but control should be easy under these conditions.

Summary

1. A canker and die-back of roses caused by *Griphosphaeria corticola* (Fckl.) v. Höhnel is described.

2. Conidial fructifications, which may be acervuli or ill-defined pycnidia, precede the formation of perithecia. Conidial isolations are of slightly differing types as regards size and shape of spores, cultural behaviour and relative pathogenicity, but at present they are all referred provisionally to *Coryneum microstictum* B. & Br. Some of the conidial isolations have given rise to perithecia, but others have not.

3. Successful inoculations both with conidia and ascospores are described.

4. In Nature, infection by this parasite may occur through pruning wounds, dead buds, leaf scars, wounds made by thorns, and rust lesions.

5. The following varieties of roses appear to be particularly susceptible to the disease: Duchess of Atholl, Lady Inchiquin and Golden Emblem.

6. The pathological anatomy of the disease is described. Infection may occur at all times of the year except summer, when the rapid formation of "gum barriers" by the host tissues checks invasion.

7. The disease is not likely to become widespread unless neglected. Diseased shoots should be cut out; with some varieties normal pruning operations should sufficiently control the disease.

We are indebted to all those who have kindly sent us material of this disease, especially Dr G. E. Deacon of Brundall, Norwich. Miss Wakefield of the Kew Herbarium and Mr W. C. Moore have assisted us greatly in the systematic part of the investigation and in the survey of the literature.

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EXPLANATION OF PLATES XIV AND XV

PLATE XIV

Fig. 1. Young canker on a stem of Kokulensky's rose stock. Infection probably occurred through a rust lesion. ×1.
Fig. 2. Cankers on a stem of a cultivated variety of rose, with conidial pustules. ×1.
Fig. 3. An acervulus-like type of fructification. ×170.
Fig. 4. A pycnidial-like type of fructification. ×170.

PLATE XV

Fig. 1. Camera-lucida drawings of *Griphosphaeria corticola*. (1) Perithecium: *cu*, cuticle, etc.; *co*, cortex; *os*, ostiole; *i*, inner part of wall; *o*, outer part of wall; *as*, ascus; *pa*, paraphysis. (2) Asci: *as*, ascus; *s*, spore; *ia*, immature ascus. (3) Portion of perithecial wall: *i*, inner part; *o*, outer part.
Fig. 2. Photograph of perithecium. ×100.
Fig. 3. Cultures of four different conidial isolations on Dox's agar.

(*Received* 3 *September* 1938)

Fig. 1.

Fig. 2.

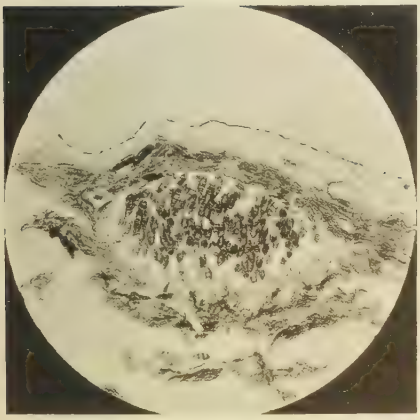


Fig. 3.

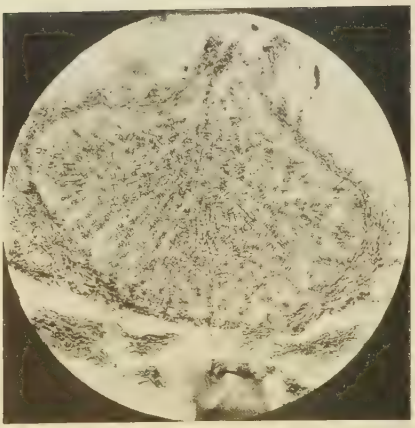
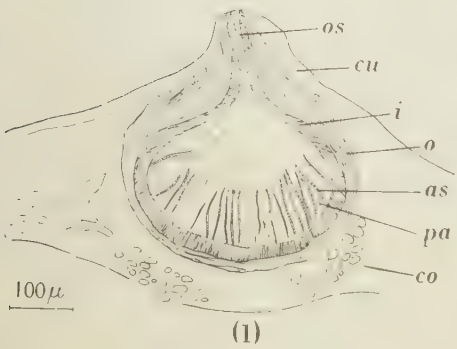


Fig. 4.

BROOKS AND ALAILY.—A CANKER AND DIE-BACK OF ROSES CAUSED BY *GRIPHOSPHAERIA CORTICOLA* (pp. 213–226)



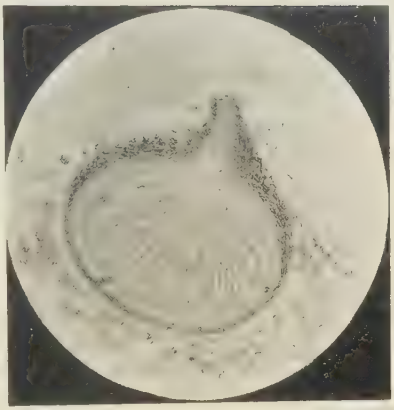


Fig. 2.

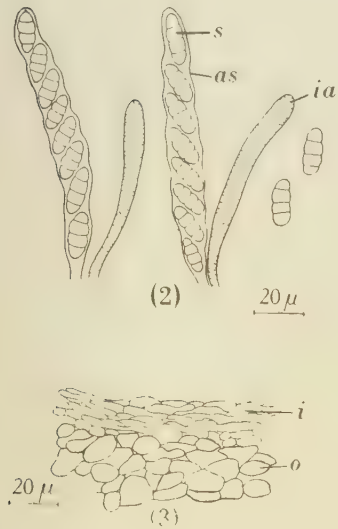


Fig. 1.

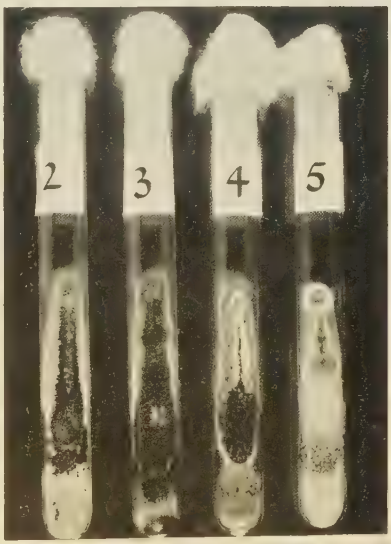


Fig. 3.

BROOKS AND ALAILY.—A CANKER AND DIE-BACK OF ROSES CAUSED BY *GRIPHOSPHAERIA CORTICOLA* (pp. 213–226)

A STUDY OF CERTAIN SPECIES OF THE GENUS *SCLEROTINIA*

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(With Plate XVI)

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Introduction

While studying *Sclerotinia serica* n.sp. (Keay, 1937) a comparative study was also made of three other species of *Sclerotinia*. Two strains of *S. Sclerotiorum* de Bary were used, one from a diseased swede and one from sclerotia on diseased hop stems. Of five strains of *S. Trifoliorum* Eriks. two were from clover (*Trifolium pratense* L.), the other three from vetch (*Vicia sativa* L.), sainfoin (*Onobrychis viciaefolia* Scop.) and a diseased carrot, the last mentioned being worthy of note since the sclerotinial rot of carrots is generally due to *Sclerotinia Sclerotiorum*. The strains are distinguished by the name of the host following that of the species. Isolations were also made from plants of *Vicia Faba* L. on which sclerotia were conspicuous. These cultures varied somewhat from those of *Sclerotinia Trifoliorum* and were markedly different from those of

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S. Sclerotiorum and *S. serica*. In May 1936 plants of *Vicia Faba*, growing near Newmarket, were seen to be rotted at or below soil level and numerous sclerotia were conspicuous. Cultures established from mycelium and sclerotia were identical with those of the first isolation, thus substantiating the view that the *Sclerotinia* attacking *Vicia Faba* is distinct from that infecting clover and other leguminous crops. Cultures established later from sclerotia from diseased beans in Oxfordshire and Wiltshire proved to be identical with those of the two previous isolations. This fungus would seem to be a variety of *Sclerotinia Trifoliorum* and has been given the varietal name *Fabae*. The different isolations are distinguished by roman numerals.

After making several consecutive cultures of *S. Trifoliorum* var. *Fabae* (I) light brown sclerotia, which failed to darken, developed in one tube. This aberrant form has remained constant although inoculated repeatedly on to lettuce and bean and reisolated.

Isolations from the pith of blackened stems of *Helianthus tuberosus* L., growing in the Cambridge Botanic Gardens, were similar in appearance to *Sclerotinia minor* Jagger. This appears to be the only record of *S. minor* occurring in Great Britain. The fungus is referred to as *S. minor* (*H. tuberosus*) to distinguish it from a culture of *S. minor* obtained from the Central Bureau voor Schimmelcultures, Baarn.

Pathogenicity

(1) *Methods*

To prevent abnormality due to prolonged culturing the fungi were inoculated at frequent intervals on to appropriate hosts and reisolated. Recent "reisolations" were used in all tests for pathogenicity, and the inocula were cut from the edge of actively growing cultures on malt extract or oatmeal agars and placed on an unwounded surface. The inoculated plants were kept under bell-jars standing in trays of water in a greenhouse. Where the organisms were pathogenic they were reisolated from the diseased tissues.

(2) *Experimental results*

The results of the experiments are summarized in Table I, the maximum infection by each fungus being indicated. The strain of *Sclerotinia Sclerotiorum* from hop was not so virulent as that from swede, but both infected the same plants with the exception of *Spergula arvensis* L. More marked differences occurred between the two strains of

Sclerotinia minor. the one from *Helianthus tuberosus* being the more pathogenic. No one strain of *Sclerotinia Trifoliorum* or of the variety *Fabae* was consistently more pathogenic than another. The variant, however, differed considerably for it attacked virulently *Gypsophila elegans* Bieb. and *Cichorium Intybus* L., which were unaffected by the parent strain, and it was more pathogenic than the normal form to *Silene gallica* L. and *S. maritima* With.

Table I. *Results of inoculation experiments*

	Cerastium arvense	*Dianthus barbatus*	*Dianthus deltoides*	*Gypsophila elegans*	*Lychnis alba*	*Silene gallica*	*Silene maritima*	*Spergula arvensis*	*Stellaria media*	*Cichorium Intybus*	*Lactuca sativa*	*Helianthus tuberosus*	*Onobrychis viciaefolia*	*Pisum sativum*	*Trifolium pratense*	*Vicia Faba*	*Vicia sativa*
S. serica	*a*	*a*	*b*	*d*	*d*	*d*	*d*	*d*	*d*	*b*	*d**	*a*	.	*c**	*a*	*b**	.
S. Sclerotiorum:																	
Swede	*b*	*d*	*a*	*d*	*d*	*c*	*d*	*b*	*a*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
Hop	.	.	.	*c*	*b*	*b*	*b*	*a*	.	*d*	*d*	*d*	*d*	*d*	*c*	*c*	*d*
S. minor:																	
Baarn	*a*	.	*a*	*c*	*a*	*a*	*c*	*a*	.	*d*	*d*	*d*	.	*d*	*a*	*a*	.
H. tuberosus	*a*	.	*b*	*d*	*c*	*c*	*c*	*c*	*a*	*d*	*d*	*d*	.	*d*	*c*	*d**	.
S. Trifoliorum var. *Fabae*:																	
I	.	.	.	*a*	*a*	*a*	*c*	*a*	.	.	*d*	*a*	*b*	*d*	*d*	*d*	*d*
II	.	.	.	*a*	*a*	*a*	*b*	*a*	.	*a*	*d*	*a*	*b*	.	.	*d*	*d*
III	.	.	.	*a*	*a*	*a*	*b*	*a*	.	*a*	*d*	*a*	*a*	.	*d*	*d*	.
IV	.	.	.	*a*	*a*	*a*	*a*	*a*	.	*a*	*d*	*a*	.	.	*d*	*d*	.
Variant	.	.	.	*d*	*a*	*c*	*d*	*a*	.	*d*	*d*	*a*	*a*	.	.	*d*	*d*
S. Trifoliorum:																	
Vetch	.	.	.	*a*	*a*	*a*	*a*	*a*	.	*a*	*d*	*a*	*d*	*d*	*d*	*d*	*d*
Sainfoin	.	.	.	*b**	*a*	*a*	*c*	*a*	.	*c*	*d*	*b*	*d*	*d*	*d*	*d*	*d*
Clover I	*a*	*a*	.	*b**	*a*	*a*	*a*	*a*	.	*b*	*d*	*a*	.	*d*	*d*	*d*	.
Carrot	*a*	.	.	*a*	*b*	*b*	*c*	*a*	.	.	*d*	*a*	.	*d*	*d*	*d*	.
Clover II	.	.	.	*b**	.	.	.	.	.	.	*d*	.	.	.	*d*	*d*	.

* Young plants.
a = Non-pathogenic.
b = Slightly pathogenic.
c = Considerable rotting.
d = Plants killed.

Little distinction can be made between *Sclerotinia Sclerotiorum* (swede) and *S. minor* (*H. tuberosus*). Although in many instances the latter rotted the plants more rapidly, both species were virulent pathogens. *S. minor* (*H. tuberosus*) was more pathogenic to several members of the Caryophyllaceae than *S. Sclerotiorum* (hop), and on *Spergula arvensis* was more pathogenic than either strain of *Sclerotinia Sclerotiorum*. On some plants *S. minor* (Baarn) behaved similarly to *S. Sclerotiorum* (hop), but on others there were considerable differences. The differences, however, between *S. minor* (Baarn) and both strains of *S. Sclerotiorum*

were no greater than those between the former and *S. minor* (*H. tuberosus*) and it was impossible to separate these two species with regard to their pathogenicity.

Comparing *S. serica* with *S. Sclerotiorum* and *S. minor* the second behaved differently from the other two on *Cerastium arvense* L. and also differed from *Sclerotinia serica* on *Dianthus barbatus* L. *Gypsophila elegans* was severely rotted by all three species, but *Dianthus deltoides* L. was attacked slightly by *Sclerotinia serica* and *S. minor* (*H. tuberosus*) only. Although *S. serica* and the more virulent strain of *S. Sclerotiorum* and *S. minor* all attacked *Lychnis alba* Mill., *Silene gallica* and *S. maritima*, the behaviour of the last two fungi varied in different experiments. *Sclerotinia serica* was markedly pathogenic to *Stellaria media* Cyrill. and *Spergula arvensis*, a common weed on light sandy soil of the type on which the original diseased *Gypsophila* plants were growing. *Sclerotinia serica* can also be distinguished by its behaviour on *Cichorium Intybus*, *Helianthus tuberosus*, *Pisum sativum* L., *Vicia Faba* and *Lactuca sativa* L. On lettuce the mycelium could only gain an entrance through the young leaves, and beans and peas were not attacked unless they were inoculated before the first foliage leaves had unfolded. It is evident that *Sclerotinia serica* differs in pathogenicity from *S. Sclerotiorum* and *S. minor*, and it is also distinguishable by the silky mycelium produced on diseased tissues and its slower rate of infection.

Excluding the variant of *S. Trifoliorum* var. *Fabae*, the pathogenicity of this fungus and that of *S. Trifoliorum* was the same, a fact of considerable practical importance. They were markedly different from *S. serica*, since they were unable to attack caryophyllaceous plants, but severely rotted the leguminous plants to which *S. serica* was only weakly pathogenic.

Records in the literature state that various plants may be infected naturally by both *S. Sclerotiorum* and *S. Trifoliorum* and Ghamrawy (1932) found no differences in pathogenicity between them. The writer's experiments showed that, by their behaviour on members of the Caryophyllaceae and on *Helianthus tuberosus* and *Cichorium Intybus*, *Sclerotinia Trifoliorum* and the variety *Fabae* were distinguishable from *S. Sclerotiorum* and *S. minor*.

On the basis of this work the fungi were classified into three groups:

(1) *S. Sclerotiorum* and *S. minor*.

(2) *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae*.

(3) *S. serica*.

The value of such data in the delimination of species is discussed later.

(3) *Infection by ascospores*

Experiments showed that the ascospores of *S. Sclerotiorum* and *S. minor* infect lettuce if they fall on yellowing leaves or on wounds caused by a hot scalpel. Infection was never initiated through a healthy leaf. That healthy tissue is infected by ascospores of *S. Trifoliorum* is suggested by the experiments of Rehm and Coleman, quoted by Wolf & Cromwell (1919). The writer found it impossible to infect clover by spraying it with an aqueous suspension of ascospores of *S. Trifoliorum*. Infection, however, resulted from apothecia developed from sclerotia buried round clover plants. When the apothecia were expanding several of the leaves and stems near ground level were dead or dying. The plants were put under bell-jars and the fungus became established on the moribund tissues and then spread to the healthy portions.

These results support the observations of other investigators that ascospores of *Sclerotinia* spp. are unable to infect healthy cuticularized tissues. In view, however, of the fact that Wilson (1937) has found that spores of *Botrytis cinerea* Pers. can penetrate adult unwounded epidermis, an ability not previously demonstrated, the whole question is in need of further study.

(4) *Infection through the soil*

It has been suggested that sclerotia of *Sclerotinia* may produce mycelium in the soil. Numerous sclerotia were buried in damp sand to obtain apothecia, but the production of mycelium by them was never observed. Since lack of detritus might have prevented such development (see Böning, 1933), whole and half sclerotia were soaked in water for 24 hr. and then buried in the soil around susceptible plants in pots. None of the plants was infected and there were no signs of mycelium having grown out from the sclerotia.

In the writer's opinion sclerotia in the soil rarely, if ever, produce mycelium and cannot be considered a source of infection by functioning in this way. That mycelium of *S. sérica* and *S. Sclerotiorum* can cause infection through the soil was shown by an experiment. Mycelial mats from liquid cultures were ground-up with sand and mixed with soil which was placed round plants of *Gypsophila elegans* and *Lactuca sativa*. Both fungi severely rotted the plants and were recovered from the diseased tissues.

Morphology

(1) *Production of apothecia*

A marked characteristic of *Sclerotinia serica* is the production of numerous apothecial stipes in cultures aged from 6 weeks to 6 months, especially those on Dox's agar. Development may begin in the dark, but they fail to mature into apothecia unless exposed to light. The majority of the stipes shrivel when a few millimetres long or the tips become swollen, urceolate and covered with microconidia, no asci developing. The production of mature apothecia could not be correlated with the age of the culture nor with the conditions of light and temperature. Apothecial stipes also arose in single-spore cultures two of which produced several mature apothecia. If the microconidia are spermatizing agents the fungus is, therefore, self-fertile. Exposing the other species in tube cultures on several media to various conditions of light and temperature was without result.

The only satisfactory method of obtaining apothecia was the following: small flower pots were partly filled with garden loam and a layer of sand 1 cm. deep; on this the sclerotia were placed, covered with sand, and watered. Large insects were excluded by wire gauze covers and the pots were generally kept outside in trays of water. Unless otherwise stated, sclerotia were not removed from the cultures until microconidia had developed.

(*a*) *Influence on apothecial production of treatment of sclerotia prior to burial.*

It was hoped to induce the rapid "germination" of sclerotia of *S. Sclerotiorum* and *S. Trifoliorum* by exposing them to different conditions of temperature and moisture before burial. None of the treatments caused more rapid apothecial development than from untreated controls and many sclerotia remained dormant. Analysis of the data suggested no explanation for this. Various investigators have stated that long periods of desiccation do not impair the "germinative" power of sclerotia of these two species. In three instances apothecia of *S. Trifoliorum* developed from sclerotia buried after storage in dry sand for nine weeks. Sclerotia stored in envelopes for 5–14 months did not produce apothecia; the same was true for *S. Sclerotiorum*.

(*b*) *Influence on apothecial production of the temperature at which sclerotia were formed.*

Sclerotia were buried immediately after removal from cultures employed in experiments on temperature relations. No apothecia developed of *S. serica*, *S. minor* (Baarn) and the variant of *S. Trifoliorum* var. *Fabae*. Sclerotia of *S. Sclerotiorum* formed at 5, 10, 15 and 20° C. produced apothecia. No microconidia had been found in the cultures at 10 and 15° C. when the sclerotia were removed. That no apothecia developed from sclerotia formed at 25° C. agrees with the observation of Kheswalla (1934). Subsequently, numerous sclerotia formed at this temperature were buried without result. Apothecia of *S. Trifoliorum* var. *Fabae* (I) arose from sclerotia formed at 0, 10 and 20° C. and from those of *S. Trifoliorum* formed at 5, 10, 15, 20 and 25° C. In view of the results obtained with *S. Sclerotiorum* it was interesting that sclerotia of *S. Trifoliorum* formed at 25° C. produced apothecia for, whereas the former grew rapidly at 25° C., the latter grew slowly, staled badly and produced smaller sclerotia than at 20° C. Sclerotia of *S. minor* (*H. tuberosus*) formed at 5, 10, 15, 25 and 27° C. produced apothecia. No explanation can be given for the failure to "germinate" of those formed at 20° C. Despite gaps in the evidence it would seem that sclerotia formed over a wide range of temperature can produce apothecia.

(*c*) *Production of apothecia by single-spore lines.*

No apothecia developed from buried sclerotia from single-spore lines of *S. Trifoliorum* var. *Fabae* and *S. serica*. Sclerotia from ten single-spore lines of *S. minor* (*H. tuberosus*) produced apothecia. Apothecia were also obtained from six single-spore lines of *S. Trifoliorum* (clover I). Seven single-spore lines were begun from one of these apothecia; when the sclerotia were buried four of the lines produced apothecia, thus showing that spores derived from a single-spore line are in no way unusual in their nuclear composition. The majority of sclerotia from single-spore lines of *S. Sclerotiorum* rotted, but three lines produced apothecia, showing that the fungus is homothallic.

(*d*) *General conclusions on apothecial production.*

General conclusions regarding apothecial production are based on the results of numerous observations during 1934, 1935 and 1936 in addition to those detailed above.

Apothecia of *S. minor* (*H. tuberosus*) were produced from June to December, fewer developing as the weather became colder. The only apothecia which appeared during the first half of the year were in pots in the greenhouse. The sclerotia did not survive more than one year. *S. minor* (Baarn) never produced apothecia.

The sclerotia of *S. serica* frequently decayed rapidly and, although many sclerotia and pieces of plectenchyma were buried, comparatively few apothecia arose. Apothecia developed in April, May, September and October so that extremes of temperature are apparently unfavourable for their development. Twice sclerotia "germinated" very quickly; in April 1935, sixteen apothecia developed from sclerotia and plectenchyma buried 36 days previously and, in May, thirteen apothecia arose from sclerotia and plectenchyma buried 18 days before.

Records in the literature show that the development of apothecia of *S. Trifoliorum* is generally in the autumn, but that it may occur until March if the weather is mild and damp. In the present work apothecia were produced from June to December, the majority appearing in October. Some sclerotia "germinated" a few months after burial, others remained dormant for nearly two years.

Apothecia of *S. Trifoliorum* var. *Fabae* developed at the same time as those of *S. Trifoliorum*, although in 1937 a few of the former developed in February and April. Many sclerotia decayed rapidly, others "germinated" after 15 months' burial. The variant of this fungus never produced apothecia.

Under natural conditions the apothecia of *S. Sclerotiorum* are recorded as developing in spring and early summer. In these experiments no apothecia developed before April. During 1935 apothecia appeared from April till December, the majority in June, and in 1936 from May to November. Sclerotia "germinated" after 30 months' burial.

Temperature and moisture are important factors influencing the "germination" of sclerotia. In these experiments, since the sand was continuously moist, temperature is assumed to be the more important factor. Although apothecial production by *S. Trifoliorum* and the variety *Fabae* showed slightly more connexion with season than that by *S. Sclerotiorum* and *S. minor*, it took place in all four fungi over a wide temperature range. The time of development was independent of the medium on which, or the temperature at which, the sclerotia were formed, or the time at which they were buried.

(2) *Size, shape and colour of apothecia*

The previous nutrition of sclerotia of *S. minor* (*H. tuberosus*) was found to affect the size of the apothecia. Numerous apothecia arose from sclerotia formed on sterilized potato, carrot and artichoke, and 104 had an average diameter of 3·9 mm. and a range of 1·5–9 mm. Seventy-three apothecia, developed from sclerotia from single-spore cultures on malt extract, had an average diameter of 2·2 mm. and a range of 1·5 mm., sixty-two of them being less than 3 mm. in diameter. Twenty-six apothecia of the original isolate grown on the same medium had an average diameter of 1·7 mm. and a range of 1·3 mm., only four apothecia being greater than 2 mm. in diameter. Since the apothecia from sclerotia formed on sterilized vegetables and those from single-spore cultures on malt developed concurrently, the difference in size was apparently due to the conditions under which the sclerotia had formed.

It is unfortunate that *S. minor* (Baarn) did not produce apothecia. Jagger (1920) gives the diameter of the apothecium as 0·5–2 mm., figures which approximate to those for *S. minor* (*H. tuberosus*) from sclerotia produced on malt. One cannot say, however, that the size of the apothecium from sclerotia grown on malt is more "typical" of the strain than that from sclerotia formed on vegetables. It would seem, therefore, that the size of the apothecial disk is of little value for distinguishing these species. This opinion is strengthened when measurements of apothecia of the other species are studied (Table II).

Table II. *Dimensions of the disks of apothecia*

Name of fungus	Average diameter of apothecia in mm.	Range in mm.
S. Trifoliorum	3·9	2 –8·5
S. Trifoliorum var. *Fabae*	2·8	2 –6·5
S. Sclerotiorum	4·3	1·5–8
S. serica	2·5	2 –5

The length of the stalk of the apothecium depends on the depth at which the sclerotium is buried and, consequently, this measurement should not be used as a diagnostic character.

Although the diameter of the disk of the apothecium is valueless for distinguishing these species, the shape and colour of the apothecia are distinctive. Since *S. serica* has already been described (Keay, 1937) it will not be dealt with here. The apothecia of *S. Sclerotiorum* are light brown; they usually develop slightly above the level of the sand and are somewhat trumpet shaped; the central depression is well marked in

young apothecia and is rarely entirely absent in mature disks. Young apothecial stalks of *S. minor* (*H. tuberosus*) are slender and of a uniform colour. In the early stages of development the edges of the tubular tip are inrolled but, as the tip increases in size, the mouth enlarges and the tubular structure becomes more marked; at the same time the stalk darkens and, in the mature apothecium, it is brown while the disk is light buff. As the disk matures it becomes flattened, the centre being slightly raised. Apothecia of *S. Trifoliorum* are distinguishable from those of the other species by their dark colour and tubular shape for, even when mature, there is generally a deep central depression. Frequently the stalk is swollen beneath the disk, gradually increasing in diameter from above the sand level and bearing the cap well above the surface. The apothecia of *S. Trifoliorum* var. *Fabae* are also produced well above the surface and are the same colour as those of *S. Trifoliorum*. The stout stalks are of a uniform width, not being swollen beneath the disks as those of *S. Trifoliorum*, and the disks of the variety *Fabae* tend to be flatter than those of the normal strains. Frequently, however, it was difficult from a cursory examination to distinguish the apothecia of these two fungi.

(3) *Measurements of ascospores and asci*

Measurements of ascospores and asci were made by means of a Leitz Okular Shrauben-mikrometer. A statistical analysis was made of the results, which are presented in Tables III and IV. More than one sample of ascospores and asci of each species was measured. All the measurements of *S. Sclerotiorum* were of the strain from swede. The strain of *S. Trifoliorum* measured was that from clover. Three of the isolations of *S. Trifoliorum* var. *Fabae* were measured. The method of collecting ascospores was to place a sterilized cover-slip, on which was a drop of sterile distilled water, over a discharging apothecium and it is presumed that only mature ascospores were collected. It was more difficult to judge the state of maturity of an ascus, but only those were measured in which the outlines of the eight spores were clearly visible. The values of the means derived from different samples of the same fungus were not identical and the sample with the greatest mean length did not always have the greatest mean breadth. Fisher's "t" test (1936) showed that in some instances a significant difference occurred between the mean value for both length and breadth, while in others only one of these values was significantly different. The cause of these variations in spore and ascus size could not be determined. For example, two samples, each

of 100 spores, of *S. Trifoliorum* were measured on the same day from two apothecia which had developed in the same pot from sclerotia formed under apparently identical cultural conditions. The means obtained, $15{\cdot}09 \times 9{\cdot}8\mu$ and $14{\cdot}52 \times 7{\cdot}62\mu$, are significantly different. On the other hand, the means $18{\cdot}92 \times 9{\cdot}34\mu$ and $18{\cdot}88 \times 10\mu$ for two samples of spores

Table III. *Dimensions of ascospores of four species of* Sclerotinia. *Each sample contained* 100 *spores, except* (i) = 50; (ii) = 35

Name of fungus	Origin of sclerotia from which apothecia developed	Range μ	Length Mean μ	Length S.E. μ	Length S.D. μ	Breadth Mean μ	Breadth S.E. μ	Breadth S.D. μ
S. Sclerotiorum (swede)	Produced in culture	10–14 by 5–8	11·84	0·08	0·8	6·52	0·05	0·503
	From a rotten carrot (i)	8–15 by 5·5–8	12·44	0·18	1·28	6·63	0·062	0·44
	Produced in culture	10–16 by 4·5–8·5	12·86	0·118	1·177	6·64	0·087	0·875
S. minor (*H. tuberosus*)	Produced on carrot	10–19 by 6–9	14·1	0·166	1·66	7·87	0·06	0·596
	Produced on malt	10–18·5 by 5·5–10	14·1	0·129	1·295	7·89	0·083	0·827
S. Trifoliorum (clover I)	Produced in culture	8·2–20 by 5–10	14·52	0·216	2·16	7·62	0·11	1·11
	As above. Another apothecium in same pot	11·5–20 by 6·5–13	15·09	0·183	1·83	9·8	0·14	1·4
	Produced in culture	10–18·6 by 4·6–12	15·4	0·19	1·94	8·86	0·158	1·58
	Produced in a single-spore culture	11·5–20 by 6–10	15·9	0·183	1·827	8·4	0·097	0·97
S. Trifoliorum var. *Fabae*:								
Isolate I	Produced in culture	12–25·2 by 6·4–14	18·7	0·25	2·5	9·15	0·20	2·0
	Produced on malt at 10° C. (ii)	14·2–23·8 by 6·8–14	18·88	0·28	1·68	10·0	0·32	1·88
	Produced on malt at 20° C.	14·4–27·4 by 7–13·7	18·92	0·199	1·99	9·34	0·158	1·58
Isolate II	From diseased beans in nature (i)	10–28·2 by 8–15·2	19·84	0·471	3·33	11·32	0·262	1·856
Isolate IV	Produced in culture (i)	11·6–24·8 by 7·4–13·2	18·76	0·379	2·68	10·04	0·284	1·47
S. serica	Produced in culture	15–26 by 7–13	21·27	0·24	2·43	9·86	0·12	1·22
	Apothecium developed in tube culture on Dox's agar	14–26·6 by 6·8–17·2	20·04	0·282	2·82	10·38	0·179	1·785

of *S. Trifoliorum* var. *Fabae* (I) are not significantly different, although the parent sclerotia had formed in cultures at different temperatures and the time of burial and "germination" was different. The sample of asci of *S. Trifoliorum* with the smaller mean length and breadth arose from a single-spore line, but the ascospores in this apothecium had a greater mean length than those of the original strain. The largest ascospores and asci of *S. Trifoliorum* var. *Fabae* were produced by an apothecium which

arose from sclerotia gathered from diseased beans in nature. In view of the influence of the nutrition of the sclerotia of *S. minor* (*H. tuberosus*) on the size of the apothecia, it is interesting that there was no difference in the size of spores and asci from sclerotia formed on malt extract and sterilized vegetables.

Variation in the size of asci and ascospores of *S. Sclerotiorum* has been described by Ramsey (1925) and Antokolskaya (1932) and the measurements given by Mundkur (1934) and Kheswalla (1934) for two strains in

Table IV. *Dimensions of asci of four species of* Sclerotinia. *Each sample contained* 100 *asci, except* (i) = 75; (ii) = 68; (iii) = 60; (iv) = 93

Name of fungus	Origin of sclerotia from which apothecia developed	Range	Length			Breadth		
			Mean	S.E.	S.D.	Mean	S.E.	S.D.
		μ	μ	μ	μ	μ	μ	μ
S. Sclerotiorum (swede)	Produced in culture	108–150 by 8·8–14·6	122·6	0·91	9·13	11·7	0·14	1·4
	Produced in culture	108–152 by 6·5–10·5	128·8	1·006	10·06	8·6	0·089	0·89
S. minor (*H. tuberosus*)	Produced on artichoke	128–182 by 9·5–15·4	149·6	1·19	11·93	12·84	0·165	1·65
	Produced on malt	130–180 by 8–14·5	150·1	1·06	10·57	12·0	0·16	1·6
S. Trifoliorum (clover I)	Produced in a single-spore culture (i)	147–191 by 9·3–15·6	165·2	0·95	8·22	12·7	0·152	1·32
	Produced in culture	160–234 by 9·8–16·5	195	1·67	16·66	14·4	0·168	1·68
S. serica	Produced in culture	155–201 by 11–22	178·3	0·97	9·73	16·8	0·227	2·27
	Apothecium developed in tube culture on Dox's agar (ii)	180–250 by 11–21	209·7	1·94	16·0	16·03	0·229	1·896
S. Trifoliorum var. *Fabae*:								
Isolate I	Produced in culture	167–232 by 10–21	199·6	1·35	13·51	16·0	0·209	2·095
	Produced in culture	165–252·5 by 10·75–20·5	200	1·67	16·67	16·29	0·236	2·36
Isolate II	From diseased beans in nature (iii)	195–248·6 by 13·7–20·5	223·4	1·89	14·68	16·96	0·194	1·50
Isolate IV	Produced in culture (iv)	177·4–249·6 by 13·7–22·4	219·2	1·525	14·71	18·23	0·156	1·507

India are considerably different. Considering the data in the literature and the results of the present investigations one has to decide whether such measurements are of any value for distinguishing species. In the writer's opinion such data can be of value, but in describing *S. Sclerotiorum* and *S. Trifoliorum* some authors have given only the range of ascus and ascospore size. The following example shows that this is inadequate. A sample of spores of *S. minor* (*H. tuberosus*) ranged in length from 10 to 18·5μ, one of *S. Trifoliorum* from 10 to 18·6μ, and it

might be thought that the spores had been produced by the same *Sclerotinia*. On analysing the data statistically, however, the means are 14·1 and 15·4μ respectively, and the difference between them is significant.

It may be seen (Table III) that for each fungus the mean values for the different samples of ascospores arrange themselves round a central value. The values in Table V are the averages of the means and show that the fungi differ considerably in their spore size.

Table V. *Average dimensions of ascospores of four species of* Sclerotinia

Name of fungus	Size of ascospores μ
S. Sclerotiorum (swede)	12·38 × 6·59
S. minor (*H. tuberosus*)	14·1 × 7·88
S. Trifoliorum (clover I)	15·23 × 8·17
S. Trifoliorum var. *Fabae* (I, II and IV)	19·02 × 9·97
S. serica	20·65 × 10·12

Moreover, Fisher's "t" test showed that the mean length of the spores of all the fungi differed significantly with the exception of *S. minor* 14·1μ, *S. Trifoliorum* 14·52μ and *S. Trifoliorum* var. *Fabae* 19·84μ and *S. serica* 20·04μ. The values of the means for breadth overlapped so much that the "t" test was not applied.

The data on ascus size in Table IV show that the values of the means for breadth overlap somewhat and that the mean length for *S. serica* overlaps considerably with those for *S. Trifoliorum* and the variety *Fabae*. Moreover, the variation in ascus size between two samples of the same fungus is more marked than that for the ascospores. The values derived by averaging the means for each fungus are given in Table VI, in which the differences between the fungi are more pronounced.

Table VI. *Average dimensions of asci of four species of* Sclerotinia

Name of fungus	Size of asci μ
S. Sclerotiorum (swede)	125·7 × 10·15
S. minor (*H. tuberosus*)	149·8 × 12·42
S. Trifoliorum (clover I)	180·1 × 13·55
S. serica	193·98 × 16·41
S. Trifoliorum var. *Fabae* (I, II and IV)	210·54 × 16·87

On applying the "t" test the means were all found to differ significantly, but this was also true when samples of the same fungus were compared, with the exception of the two sets of asci of *S. minor*.

Although the ascospores and asci of each fungus have a considerable range in size, on the whole, those of any one organism are distinct from those of the other four. It seems justifiable, therefore, to consider that the five organisms in question are distinct.

Cultural behaviour

(1) *Temperature relations*

A study was made of the influence of temperature upon the rate of the lateral extension of the mycelium of the different organisms on 5% malt-extract agar in Petri dishes. The minimum, maximum, and optimum temperatures and the time taken by the cultures to reach a size of 6 cm. diameter at 20° C. are given in Table VII. In this work the optimum temperature is that temperature at which the value of the velocity rate of the reaction is highest throughout the whole of the experimental period, i.e. the temperature at which the time for the fungus to reach various diameters is at a minimum throughout the experimental period. The minimum and maximum temperatures are the lowest and highest at which there was any measurable growth.

Table VII. *Temperature relations of four species of* Sclerotinia

Name of fungus	Range ° C.	Optimum ° C.	Time, in hr., to reach a diameter of 6 cm. at 20° C.
S. Sclerotiorum (1)	0–30	25	40
,, (2)	0–30	20	36·5
S. minor (*H. tuberosus*) (1)	0–30	25	42
,, (2)	0–30	25	36·5
S. minor (Baarn) (1)	5–30	20	47
,, (2)	5–30	20	45
S. Trifoliorum (1)	0–27	20	69
,, (2)	0–27	20	65·5
S. Trifoliorum var. *Fabae**	0–27	20	59·5
,, variant	0–30	25	42
*S. serica**	0–25	20	103

* Average of three experiments.

Column 1 (Table VII) shows that, with the exception of *S. minor* (Baarn), all the cultures made a measurable amount of growth at 0° C. *S. Trifoliorum* and the variety *Fabae*, however, were the only two organisms which continued spreading until the whole of the medium was covered; cultures of the former took 27 days to reach a diameter of 9·1 cm., of the latter 22 days. *S. Sclerotiorum*, *S. minor* (*H. tuberosus*) and the variant of *S. Trifoliorum* var. *Fabae* all ceased to spread at 0° C. after varying periods ranging from 6 to 19 days. In all instances the rate of spread was small, the greatest being by *S. minor* (*H. tuberosus*) which took 10 days to reach a diameter of 1·67 cm. The diameter of cultures of *S. serica* was 4·7 cm. before the mycelium ceased to spread, but the rate of advance was slow as it took the cultures 23 days to reach this size.

S. serica had the lowest maximum temperature, 25° C. That for *S. Trifoliorum* and the variety *Fabae* was 27° C. The variant of the latter fungus, however, resembled *S. Sclerotiorum* and *S. minor* in having a maximum of 30° C. In every instance the rate of spread at the maximum temperature began at a fairly high level and then fell rapidly, and the cultures did not enlarge in diameter after the first three or four days.

The optimum temperature for *S. Sclerotiorum* was different in two experiments. At 25° C. both sets of cultures took 37·5 hr. to reach a diameter of 6 cm.; comparing these values with those in column 3 (Table VII) it will be seen that there was little difference between the rates of spread at 20 and 25° C. The strain of *S. minor* from Baarn had a lower optimum temperature than that from *H. tuberosus*. *S. serica*, *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae* all had an optimum of 20° C. In this feature also the variant of the latter fungus differed from the parent strain as the optimum temperature was 25° C.

The values in column 3 (Table VII) indicate the rate of spread of the mycelium. *S. Sclerotiorum* and *S. minor* (*H. tuberosus*) spread very rapidly; *S. minor* (Baarn) also spread quickly, though consistently slower than the two former fungi. *S. Trifoliorum* and the variety *Fabae* spread more slowly. The variant of the latter differed from the parent strain, equalling in rate *S. Sclerotiorum* and *S. minor* (*H. tuberosus*). *S. serica* was easily distinguishable from the other organisms as the rate of spread was much slower.

Summarizing the results the fungi fall into four groups:

(1) *S. serica*.
(2) *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae*.
(3) *S. minor* (Baarn).
(4) *S. minor* (*H. tuberosus*), *S. Sclerotiorum* and the variant of *S. Trifoliorum* var. *Fabae*.

The fact that the variant which arose from *S. Trifoliorum* var. *Fabae* has such different temperature relations from the parent form might seem to negative the use of such data in the delimitation of species, but this argument is perhaps not legitimate in the present instance. The same conclusion, nevertheless, must be arrived at if one considers the behaviour of the other fungi. *S. Sclerotiorum* and *S. minor* (*H. tuberosus*) are very similar in their relation to temperature although their appearance in culture is quite distinct, their apothecia are of different shape and colour and they produce asci and ascospores, the mean length and breadth of which are different. Also, although the two forms of *S. minor*

could not be separated systematically on morphological grounds, the differences between them with regard to the influence of temperature are greater than those between *S. minor* (*H. tuberosus*), *S. Sclerotiorum* and the variant of *S. Trifoliorum* var. *Fabae*.

(2) *Appearance in culture*

The appearance of the fungi in culture on 5% malt-extract agar may be seen in Pl. XVI, figs. 1–6. For a description of the cultural appearance of *S. serica* see Keay (1937). On all the media employed *S. Sclerotiorum* produces more mycelium than *S. Trifoliorum* and the sclerotia of the former are covered by a fine mycelial weft. On oatmeal agar *S. Trifoliorum* stains the medium brown and produces small waxy globules of microconidia; these waxy bodies have never been observed in a culture of *S. Sclerotiorum*. In test-tube cultures on 5% malt-extract agar *S. Trifoliorum* produces haptera which stretch from the edge of the medium to the glass, often having the appearance of a folded fan, with the end flattened against the glass. Frequently microconidia develop on these in great abundance. As well as these haptera a delicate black membrane is often developed on the surface of the glass. Cultures of *S. Sclerotiorum* do not produce these structures.

The form of *S. minor* from *H. tuberosus* produces smaller sclerotia and more fluffy mycelium than the form from Baarn, in which the mycelium is somewhat flocculated. Chivers (1929) found that the sclerotia of *S. minor* decreased in size with decreasing temperature. This was so for the form from *H. tuberosus*, but not for the form from Baarn, in which the size of the sclerotia was little influenced by temperature. On Dox's medium, oatmeal and potato-dextrose agars the cultures exhibited the same differences, the sclerotia of the Baarn form showing a tendency to coalesce. The cultures were indistinguishable, however, on sterilized carrot, potato and artichoke, each producing a continuous sclerotial crust. On all the media used the appearance of these two fungi is quite distinct from that of the other species.

S. Trifoliorum var. *Fabae* resembles the normal form of *S. Trifoliorum* in that the mycelium on all solid media is inconspicuous, and both fungi produce small, waxy globules of microconidia on oatmeal agar. The variety from bean, however, has smaller sclerotia and does not produce the conspicuous haptera which develop in cultures of the strains from clover. Cultures of both fungi grown on liquid carrot extract were indistinguishable.

The variant of *S. Trifoliorum* var. *Fabae* was first noted on account of the pale colour of the sclerotia. The coloration varied according to temperature; at 5° C. they were light cream, at 25° C. light brown. The sclerotia also differ from those of the parent in being larger and smoother, and the cultures produce more aerial mycelium.

Classifying the fungi with regard to their appearance in culture the following groups emerge:

(1) *S. Sclerotiorum* (hop and swede).
(2) *S. minor* (Baarn and *H. tuberosus*).
(3) *S. serica.*
(4) (*a*) *S. Trifoliorum* (clover, vetch, sainfoin, and carrot); (*b*) *S. Trifoliorum* from *Vicia Faba.*
(5) Variant of fungus from *V. Faba.*

DISCUSSION

In deciding upon the systematic position of the fungi studied their pathogenicity has not been considered, as evidence from this source is valueless for differentiating species. The ability of a fungus to parasitize a plant is essentially due to the fact that it can use substances in the host tissue for its own metabolism and two fungi which have entirely different life histories may use the same basic plant substances for their nutrition. Further, it was found possible to modify the pathogenicity of the fungi, for when grown on Dox's agar their pathogenicity was greatly reduced. Evidence, however, derived from both morphological and cultural studies has been taken into account.

A study of the literature revealed no reason for suspecting that the isolates from hop and swede were not *S. Sclerotiorum* and those from clover, carrot, sainfoin, and vetch were not *S. Trifoliorum.* These two fungi have long been considered distinct species and these investigations support this view, for they have demonstrated that the fungi can be distinguished by the structure of their apothecia, the size of their ascospores and asci, and their cultural characteristics.

The morphology and cultural appearance of *S. serica* are different from those of all the other isolates. Since no published description was found which would apply to this isolate it was decided that it was a previously undescribed species. This seemed justifiable since, in the writer's estimate, the degree of difference between this fungus and *S. Sclerotiorum*, *S. minor*, and *S. Trifoliorum* was as great as that between the three latter fungi, which are generally regarded as three distinct species.

The morphology and cultural behaviour of the *Sclerotinia* from *Helianthus tuberosus* are distinct from those of *Sclerotinia Sclerotiorum*, *S. Trifoliorum* and *S. serica*. Although this isolate differed somewhat from a culture of *S. minor* obtained from Baarn, the measurements of asci and ascospores of the former agree well with those made by Jagger (1920). Moreover, Chivers (1929) showed that six strains of this fungus isolated from different plants in widely separated geographic regions displayed differences in their cultural characteristics. There seems no reason then why the fungus from *Helianthus tuberosus* should not be considered a form of *Sclerotinia minor*.

Jagger (1920) was the first to describe the *Sclerotinia* producing small sclerotia as a separate species and it has been accepted as such by many workers. In Cunningham's (1927) view, however, it is a form of *S. Sclerotiorum*, as the differences cannot be "considered as possessing specific value". The writer has shown that the fungus known as *S. minor* is quite distinctive in morphology and cultural characters from *S. Sclerotiorum*. If the view be held that *S. minor* is only a form of *S. Sclerotiorum*, then it would seem to be necessary to regard the two fungi *S. Trifoliorum* and *S. serica* as different forms of the same species.

Considered from a purely morphological point of view it is possible that the isolate from *Vicia Faba* might be regarded as a distinct species. Cultural characteristics, however, should also be taken into account. It is not easy to define a standard whereby the degree of difference between two cultures can be judged, and such comparisons therefore depend on the opinion of the investigator. In comparing cultures of *Sclerotinia Trifoliorum* and the isolate from bean the differences are quite apparent, but it is doubtful whether they are any greater than the differences between the two forms of *S. minor*. The writer considers that it is not justifiable to regard the isolate from bean as a species distinct from *S. Trifoliorum*. Its distinguishing characters are, however, so constant that it is considered to be a variety. The association of the fungus with beans, further evidence of which association has been obtained since the conclusion of the work described above, led to it being given the varietal name of *Fabae*.

Summary

1. Investigations were carried out on *Sclerotinia serica*, two strains of *S. Sclerotiorum* de Bary, five strains of *S. Trifoliorum* Eriks., two forms of *S. minor* Jagger, four isolations of a *Sclerotinia* from *Vicia Faba* considered to be a variety of *Sclerotinia Trifoliorum*, and a variant which arose from one of these isolations.

2. Their pathogenicity to several different plants was tested and the fungi were found to fall into three groups: (1) *S. Sclerotiorum* and *S. minor*, (2) *S. Trifoliorum* and its variety *Fabae*, and (3) *S. serica*.

3. A variant with light brown sclerotia which arose from *S. Trifoliorum* var. *Fabae* differed from the parent form in pathogenicity.

4. Ascospores of *S. Sclerotiorum*, *S. minor* and *S. Trifoliorum* did not infect healthy leaves.

5. The production of mycelium by sclerotia in the soil was not observed.

6. *S. serica* differed from the other fungi in that it produced numerous apothecial stipes and several apothecia in test-tube cultures.

7. Exposing sclerotia to different conditions of temperature and moisture after their removal from culture and before their burial in damp sand did not hasten apothecial production.

8. Sclerotia formed in cultures grown over a wide temperature range produced apothecia when subsequently buried in damp sand.

9. Single-spore lines of *S. serica*, *S. Sclerotiorum*, *S. minor* and *S. Trifoliorum* produced apothecia.

10. Apothecial production took place over a wide temperature range.

11. Apothecia of one form of *S. minor* arising from sclerotia formed on sterilized vegetables were larger than those from sclerotia formed on malt-extract. The dimensions of the apothecia are given and a description of the apothecia of *S. Sclerotiorum*, *S. minor*, *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae*.

12. Measurements are given of asci and ascospores.

13. The minimum, maximum and optimum temperatures for linear spread of mycelium are given: also the time taken by cultures to reach a diameter of 6 cm. at 20° C. The view is expressed that such data should not be used for differentiating species. *S. serica* spread more slowly than the other species. The variant of *S. Trifoliorum* var. *Fabae* had different temperature relations from the parent form.

14. A brief description is given of the cultural appearance of *S. Sclerotiorum*, *S. Trifoliorum*, *S. minor*, *S. Trifoliorum* var. *Fabae* and its variant.

15. The classification of the fungi is discussed.

This work was carried out under the supervision of Prof. F. T. Brooks, to whom I wish to express my sincere thanks for suggesting the problem and for the interest which he showed in its progress. I am grateful to Dr Kidd for permission to use the constant temperature

chambers at the Low Temperature Research Station. I also wish to thank Dr Dillon-Weston, Dr Pethybridge and Dr A. Smith for sending me material.

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EXPLANATION OF PLATE XVI

Fig. 1. Culture of *Sclerotinia minor*, strain from Baarn.
Fig. 2. Culture of *S. serica*.
Fig. 3. Culture of *S. minor*, isolated from *Helianthus tuberosus*.
Fig. 4. Culture of *S. Sclerotiorum*.
Fig. 5. Culture of *S. Trifoliorum* var. *Fabae*.
Fig. 6. Culture of *S. Trifoliorum*.

All cultures grown on 5% malt-extract agar at 20° C.

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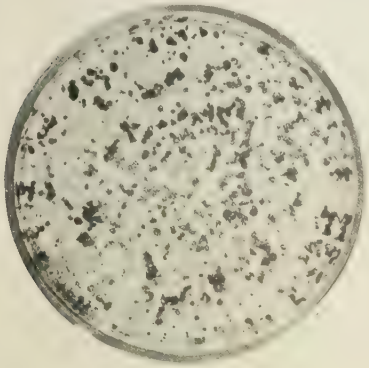

Fig. 1.

Fig. 2.

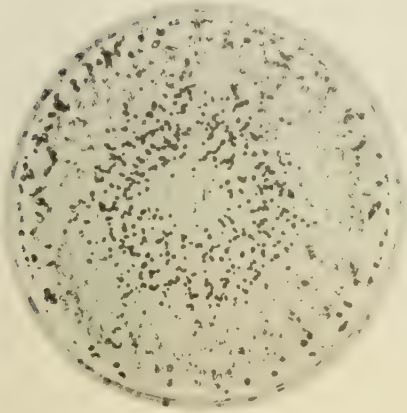

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

KEAY.—A Study of Certain Species of the Genus *Sclerotinia* (pp. 227–246)

INTERACTION OF SOIL MICRO-ORGANISMS WITH *OPHIOBOLUS GRAMINIS* SACC., THE FUNGUS CAUSING THE TAKE-ALL DISEASE OF WHEAT

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(With 2 Text-figures)

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Introductory

The literature relating to the interaction of micro-organisms in the soil is extensive and has recently been reviewed by Waksman (1937). With regard to the effect of other soil organisms upon *Ophiobolus*, the outstanding contributions are those of Simmonds (1928), Sanford & Broadfoot (1931), Henry (1932), Broadfoot (1933*a*, 1933*b*) and Garrett (1934, 1936, 1937, 1938). It has been shown that the pathogenicity of *Ophiobolus* is greater in sterilized than in unsterilized soil and greater in sand than in loam. These effects are explained on the basis of soil factors, in particular of antagonism by micro-organisms. The same antagonism has also been adduced to explain the more or less rapid disappearance of active *Ophiobolus* mycelium from a contaminated soil.

In the present work an attempt has been made to evaluate the part played by particular soil organisms in restricting the parasitic power of *Ophiobolus* and to study the principles concerned. The examination was restricted to those organisms which appear as contaminants in isolations

of *Ophiobolus* from infected roots. If it be true that certain organisms determine the parasitism of *Ophiobolus*, one would expect to find them at one time or another in association with the pathogen.

Experimental

(1) *Soil organisms as determining the recovery of* Ophiobolus *mycelium from the roots of inoculated wheat seedlings*

Ophiobolus inoculum makes little or no growth in unsterilized soil. However, under such conditions the inoculum infects the young roots if wheat seed is planted on it, the fungus growing along the roots but as a rule not being recoverable by the plating method after the lapse of some time. The infected roots soon become so rotted by the invasion of secondary organisms that the presence of *Ophiobolus* is hard to establish. It is of interest to determine how long the fungus remains alive in the host tissue itself and what organisms follow. If the soil organisms influence the disintegration of *Ophiobolus* it is those which emerge in association with the pathogen from infected roots at one stage or another that probably take a prominent part in the destruction of the parasite.

A number of representative soil types were used in these experiments. These were as follows:

(1) Richmond Deer Park, *p*H 4·8—a sandy heath soil.
(2) Jersey St Mary, *p*H 5·0—a potato soil.
(3) Sand, *p*H 6·6.
(4) Slough, *p*H 6·7—a medium loam.
(5) Cambridge, *p*H 6·8—a black fen soil.
(6) Spalding, *p*H 6·9—a light loam.
(7) Chelsea Physic Garden, *p*H 7·2—a black garden loam.
(8) Ramsgate, *p*H 8·0—a light chalk soil.

Soil samples of the above were adjusted to a suitable water content by Garrett's method (1936), packed into tumblers, seeded with wheat over active inocula of *Ophiobolus* as described by Garrett (1936) and incubated at 20° C. At weekly intervals two sample tumblers were taken from each batch. The soil was removed as far as possible by washing and finally by careful brushing of the roots. The three seminal roots which had grown through the inoculum were secured and the laterals removed. A 1 cm. portion of these roots from immediately below the inoculum was cut off, thoroughly washed in running water for about an hour and sterilized by the silver nitrate method (Davies, 1935). The material was then placed on potato-dextrose agar and incubated at

25° C. At each sampling the number of roots examined varied from ten to twenty-five. The organisms appearing from the roots were examined after about 2 weeks. A record was kept of them and the percentage recovery of *Ophiobolus* noted. Similar samples were taken after 2, 3, 4, 5, 6 and 16 weeks from sowing. The treatment of these was identical with that described.

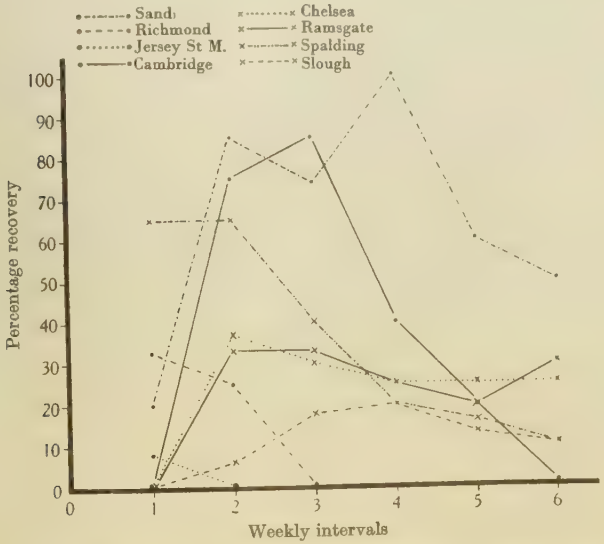

Fig. 1. Percentage frequency of recovery of *Ophiobolus* from infected wheat plants, in various soils at different intervals from inoculation.

The record of one such series of determinations is given in Fig. 1. A repeat experiment gave essentially the same result. In the figure the ordinates represent the percentage frequency of *Ophiobolus* in the various platings, i.e. the percentage of cases in which *Ophiobolus* was recovered in relation to the number of platings.

The general conclusions which may be drawn from the data of Fig. 1 may be stated as follows:

(1) In practically all cases the percentage recovery after 1 week was low. This would readily be interpreted as indicating that the first sampling took place before parasitism of the root was well established.

(2) In all the soils maximum recovery was obtained at about 2–4 weeks from inoculation.

(3) The highest percentage of recovery was obtained from sand, the lowest from acid soils. Also, while the parasite could be isolated freely from infected roots in sand after 6 weeks' attack, it had already disappeared from infected roots in the acid soils by the third week.

(4) Soils of more normal type showed an intermediate behaviour, i.e. the maximum recovery of *Ophiobolus* was lower than in sand but persistence of the parasite was greater than in acid soils. Soil alkalinity apparently favoured the persistence of the parasite.

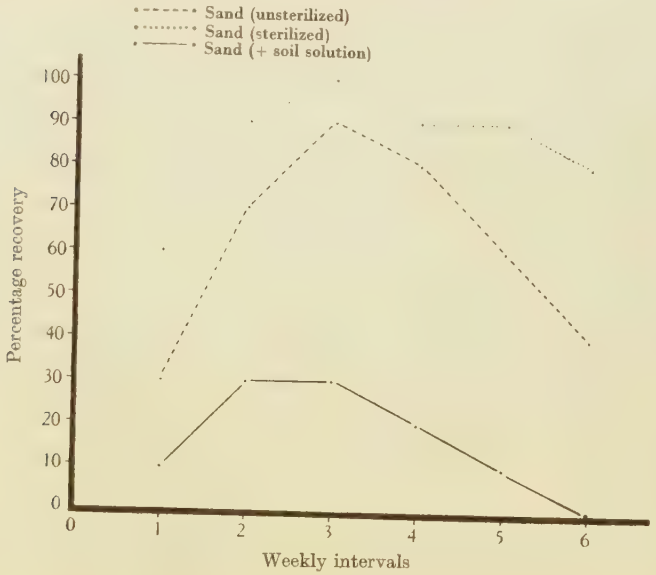

Fig. 2. Percentage frequency of recovery of *Ophiobolus* from infected wheat plants in various modifications of sand culture.

Samplings which were made 16 weeks after inoculation confirmed the above conclusions. *Ophiobolus* was recovered in 20 and 8% of the isolations from sand and Ramsgate (alkaline) soil respectively, but not from any of the others.

The relation of sand as a rooting medium to the persistence of *Ophiobolus* was further tested. A triplicate series of tumblers was set up with sand, (*a*) unsterilized, (*b*) sterilized by autoclaving, (*c*) unsterilized but watered with a soil solution obtained from Jersey St Mary soil. Sowings of wheat over active mycelium of *Ophiobolus* were then made in the manner described above. The results, expressed as previously, are shown in Fig. 2.

Fig. 2 shows that the chance of recovering *Ophiobolus* from infected roots is increased when contaminating organisms are as far as possible excluded and, conversely, that it is lessened when conditions are such as to favour microbial development.

Throughout the experiment of Fig. 1, attention was paid to the identity of the contaminating organisms which appeared in the isolation plates. Complete identification of genus and species was only occasionally possible but, in general, the genus was established. The data obtained are set out in Table I, in which the percentage frequencies of recovery of *Ophiobolus* are also inserted.

Table I. *Contaminants obtained in the isolations of* Ophiobolus *from infected roots of wheat grown in different soils*

Weekly interval	Richmond *p*H 4·8	Jersey St Mary *p*H 5·0	Sand *p*H 6·6	Slough *p*H 6·7	Cambridge *p*H 6·9	Spalding *p*H 6·9	Chelsea *p*H 7·2	Ramsgate *p*H 8·0
1	*P.*, *Py.* (33)	*B.*, *F.*, *P.*, *T.* (8)	*B.*, *M.* (20)	*B.* (0)	*X* (0)	*B.* (65)	*F.*, *T.* (0)	*X* (0)
2	*F.*, *M.*, *Py.*, *T.* (26)	*X*, *M.*, *T.* (0)	*B.* (85)	*F.*, *P.*, *T.*, *V.* (6)	*X* (75)	*X* (65)	*Asp.* (37)	*B.*, *P.* (33)
3	*T.* mainly (0)	*Asp.*, *F.*, *R.*, *T.* (0)	*Asp.*, *P.* (64)	*T.*, *X* (18)	*P.*, *T.* (80)	*T.* (40)	*B.* (30)	*Asp.*, *B.*, *P.* (33)
4	*T.*, *Py.* (0)	*T.* only (0)	*X* (100)	*P.*, *T.* (20)	*B.*, *P.* (40)	*Acr.*, *T.* (20)	*Asp.*, *B.*, *F.*, *T.* (25)	*P.* (25)
5	*T.* only (0)	*X* (0)	*Asp.*, *B.* (60)	*Bot.*, *F.*, *T.*, *X* (13)	*B.*, *Bot.* (20)	*B.*, *T.*, *X* (16)	*B.*, *X* (25)	*B.*, *P.*, *X* (20)
6	*T.* only (0)	*T.* only (0)	*Asp.*, *B.* (50)	*B.*, *P.* (10)	*Acr.*, *B.*, *V.*, *X* (0)	*B.*, *P.*, *T.* (10)	*B.* (26)	*B.* (30)

Acr. Acrostalagmus; Asp. Aspergillus; B. Bacterium; Bot. Botrytis; F. Fusarium; M. Macrosporium; P. Penicillium; Py. Pythium; R. Rhizoctonia; T. Trichoderma; V. Verticillium; X, unidentified fungi.

Figures in brackets represent the percentage recovery of *Ophiobolus*.

While it is recognized that no clear indication of microbial activity in a soil can be obtained by the plating method, certain tentative suggestions can be made from Table I. In the platings from sand and Ramsgate soil (in which the persistence of *Ophiobolus* was greatest) *Trichoderma* did not appear, whereas in platings from Richmond soil, which showed minimum persistence of *Ophiobolus*, the dominant isolate after 2 weeks was *Trichoderma*. The remaining soils show an intermediate state, i.e. among the organisms isolated *Trichoderma* was sometimes present and sometimes not. The results thus fall into line with the view which ascribes particular interest to the fungus *Trichoderma* in connexion with the disappearance of *Ophiobolus* mycelium (Weindling, 1932, 1934).

The conclusions to be drawn from the foregoing results may be stated as follows:

(1) The life of *Ophiobolus* in the host tissue is somewhat limited. It persisted longer in sand and in the alkaline soil, being recoverable even after 4 months from the time of inoculation, though the percentage recovery was then much reduced. On the other hand, it disappeared within a few weeks from inoculation in certain acid soils.

(2) Whereas the long persistence of *Ophiobolus* in the sand medium may be ascribed to the comparative absence of soil organisms from the latter, the converse relation applies to soils of the ordinary garden loam type. In particular, it is suggested that when soil conditions favour the development of the fungus *Trichoderma*, they are very unfavourable for the persistence of *Ophiobolus*.

(2) *Soil organisms as affecting the growth of* Ophiobolus *in artificial cultures and in soil*

To ascertain how far the organisms isolated as described above (*vide* Table I) exerted an antagonistic effect upon *Ophiobolus*, a series of growth experiments was carried out on agar plates and in soil.

Cultures on agar plates. Inocula of *Ophiobolus* and of a particular contaminant organism were placed at opposite sides of Petri dishes of standard diameter. The medium used was potato dextrose agar of initial *p*H 5·8. The plates were incubated at 20° C. At the time when the rate of advance of *Ophiobolus* in the direction of the contaminant was beginning to fall, the width of the clear space separating the two growths was measured. Since the rate of growth of *Ophiobolus* was known from subsidiary experiments to remain fairly constant in the absence of a contaminant, one can ascribe the retardation to a staling effect arising from the contaminant. Further, the greater the distance at which this effect is shown, the greater the antibiotic action of the contaminant concerned. One can thus arrange the contaminating organisms in a series in this respect. Arbitrarily, one can subdivide this series into three groups as follows:

(1) The most strongly antagonistic, which produce an effect on *Ophiobolus* at a distance of 1·5 cm. or more. These are: *Aspergillus* B, *Fusarium culmorum*, *Penicillium* E, H and I, *Rhizoctonia*, *Trichoderma lignorum* B and all bacterial isolates except D, G and H.

(2) Moderately antagonistic, producing an effect on *Ophiobolus* at less than 1·5 cm. and more than 0·5 cm. These are: *Acrostalagmus*

cinnabarinus, *Aspergillus* A and C, *Fusarium* sp., *Penicillium* C, D, F and G, *Trichoderma lignorum* A, *Verticillium* A and B, and Bacteria D, G and H.

(3) Least antagonistic, producing an effect only at less than 0·5 cm. distance. These are: *Botrytis cinerea*, *Penicillium* A, B, *Pythium* sp., and two unidentified fungi.

The further behaviour of such double cultures was investigated, viz. as to whether they failed to meet in the long run or whether, if they met, they intermingled or not. In this connexion one can distinguish four types:

A. Cultures not meeting, both organisms becoming staled.

B. Cultures meeting but not intermingling.

C. Cultures meeting, the contaminant growing over the *Ophiobolus* culture and preventing further growth of the latter.

D. Cultures meeting and growing through each other with very little obvious interference the one with the other.

Three isolates, *Fusarium culmorum*, *Rhizoctonia* sp. and a strain B of *Trichoderma lignorum*, produced a staling effect on *Ophiobolus* at the greatest distance (1·5 cm. or over) and finally completely overgrew the *Ophiobolus* culture and inhibited its growth (group (C) above). One may suggest therefore that, on the medium used, these organisms are the most potent competitors with *Ophiobolus* inasmuch as they check its growth without themselves being materially affected. The least antagonistic effect was shown by a species of *Pythium* which freely intermingled with the *Ophiobolus* without any retarding effect being shown by either.

How far these effects would be shown on other media is not known. A series of experiments with potato-dextrose agar adjusted to initial *p*H 2·8–3·0, 5·8 and 9·2 gave the groupings shown in Table II, the nomenclature being as above. The figures show the number of fungi which fell into each group.

Table II. *Effect of acidity of medium upon degree of antagonism*

Initial *p*H of medium	Group A	Group B	Group C	Group D
2·8–3·0	8	8	6	1
5·8	7	13	3	1
9·2	4	15	3	1

The general suggestion is that acid conditions tend to accentuate staling, e.g. by increasing the number of organisms which act against *Ophiobolus* so as to prevent the cultures from meeting. Acid conditions

also increase the number of organisms which finally grow over the *Ophiobolus* cultures.

Cultures in soil. Twenty grammes of soil were placed over filter paper in Petri dishes containing the requisite amount of water to make up the saturation to 70%. The plates were sterilized at 10 lb. for 1 hr. on three successive days and 5 c.c. of a bacterial or a fungal spore suspension sprinkled uniformly over three plates. These were then inoculated with *Ophiobolus* disks in the centre. The control plates were sprinkled with the corresponding amount of sterile water.

The following results were obtained after 10 days' incubation at 25° C.:

(1) Growth of *Ophiobolus* alone ... ... 3·56 cm.

(2) Growth of *Ophiobolus* in the presence of *Aspergillus* A and B, *Botrytis cinerea*, *Fusarium culmorum*, *Penicillium* A, B, D, E, F, H and I, *Trichoderma* A and B, *Verticillium* A and B ... Nil

(3) Growth of *Ophiobolus* in the presence of *Aspergillus* C, *Fusarium* sp., *Penicillium* C and G, *Rhizoctonia* and *Trichoderma* C ... ... 0·1–0·3 cm.

(4) Growth in the presence of two unidentified fungi 1·0–1·3 cm.

With the *Pythium* sp., which in preceding experiments showed no antagonistic action to *Ophiobolus*, no result was obtained, since the contaminant produced a continuous film over the soil surface, thereby completely obscuring the *Ophiobolus*.

The effect of the bacterial contaminants was in general less marked. Only one of these completely suppressed growth, with several (B, E, F and G) the growth of *Ophiobolus* was reduced to less than 0·5 cm., while the remainder allowed growth exceeding 1 cm.

These two methods of assessing relative antagonistic effect, one on agar plates and the other in soil, show fairly good agreement. On the whole the effects are more pronounced in the soil cultures, as would be expected from the method of inoculation adopted. It is noteworthy that nearly all the contaminant organisms were strongly repressive of *Ophiobolus* growth when inoculated into sterilized soil.

(3) *Metabolic products of soil organisms in artificial culture as affecting the growth and pathogenicity of* Ophiobolus

The effect of soil organisms on the activity of *Ophiobolus* mycelium may be ascribed either to competition for food materials, to the production of substances inimical or even toxic to the latter fungus, or to a combination of both. With a view to separating these factors, the

various contaminant fungi were cultured separately and the medium in which they had grown was tested, either as such or after modification, with regard to its effect on the growth and pathogenicity of *Ophiobolus* mycelium.

As synthetic media are not suitable for the growth of *Ophiobolus*, potato-dextrose solution with 1% dextrose was used. Flasks containing 50 c.c. of this solution were inoculated with a spore suspension of the various contaminants and incubated at 25° C. After 5 days and 21 days sample flasks were withdrawn and the fungus in each case removed by filtration through a Chamberland filter. These media were then used as follows:

A. Undiluted.
B. Diluted to one-half with distilled water.
C. Diluted to one-fourth with distilled water.
D. Diluted to two-thirds with potato-dextrose solution.
E. Undiluted but boiled for 15–20 min.

The various preparations were placed, together with controls of the original unaltered medium, in specimen tubes and each inoculated with a standard disk of *Ophiobolus* mycelium. After 5–7 days, growth was measured. The growth in the controls varied from 2·0 to 2·3 cm., and that in the various used media was as shown in Table III. The second column of the table gives the *p*H reaction of the unmodified used solution A.

The effects are thus somewhat various. Where inhibition of *Ophiobolus* is complete (group I of Table III), none of the treatments of the stale medium (cols. B, C, D, E) has, except in a few cases, caused any improvement in growth. That addition of food (col. D) does not as a rule permit of growth indicates the presence of growth-inhibiting substances. In group III of Table III, the staling effect is not shown, and none of the treatments has produced any material effect. In group II the condition is intermediate. In a few instances (e.g. *Aspergillus* A) high acidity may be the repressing factor, but there is no general rule. When 21-day-old solutions were similarly tested similar effects were shown, but the degree of inhibition was in general less pronounced.

Media staled by the various bacterial isolates gave results of the same kind as those obtained with the fungi, viz. the growth of *Ophiobolus* was either completely inhibited, retarded or unaffected. Addition of food or dilution made little difference. Boiling removed the repressive effect in one case only. The data again suggested the production of substances inhibitory to the growth of *Ophiobolus*.

Table III. *Effect of fungal metabolic products upon growth of* Ophiobolus

Organisms	*p*H	Growth of *Ophiobolus* in cm. A	B	C	D	E
		Group I. Growth nil				
Aspergillus A	2·8 or less	0·0	0·0	0·0	0·0	0·0
Bot. cinerea	6·4	0·0	0·0	0·0	0·0	Trace
Penicillium C	4·4	0·0	0·0	0·0	0·0	0·0
,, D	4·5	0·0	0·0	0·0	1·2	0·0
,, E	4·6	0·0	0·0	0·0	0·0	0·0
,, F	5·6	0·0	0·0	0·0	0·0	0·0
,, H	5·8	0·0	0·0	0·0	0·0	0·0
Trichoderma A	5·2	0·0	0·0	1·4	0·0	0·0
,, B	—	0·0	0·0	0·0	0·0	2·3
		Group II. Growth less than 1 cm.				
Penicillium I	7·3	0·9	0·0	1·0	2·0	1·6
Verticillium A	5·8	0·5	Trace	1·7	2·1	0·0
		Group III. Growth over 1 cm.				
Acrostalagmus cinnabarinus	7·2	1·9	2·1	2·0	2·1	1·8
Aspergillus B	5·2	2·3	2·3	2·3	2·3	2·3
,, C	5·7	2·3	2·3	2·3	2·3	2·3
Fusarium A	7·3	2·3	2·3	2·3	1·7	2·3
,, B	7·4	2·0	2·2	2·0	2·2	2·2
Penicillium A	7·6	2·0	1·9	2·0	2·1	2·1
,, G	6·4	1·7	2·1	1·3	2·0	0·0
Pythium	4·8	2·1	2·3	2·0	2·2	1·4
Rhizoctonia	6·4	2·3	2·3	2·3	2·3	2·3
Trichoderma C	5·6	2·0	1·9	2·1	2·3	2·3
Verticillium B	5·2	1·5	1·5	0·8	2·3	1·5

For pathogenicity tests *Ophiobolus* disks were placed for 5–7 days in the 5-day and 21-day-old filtered stale media and presoaked seeds were planted over them in tumblers containing moist sand. The seed and inocula after planting were also covered with sand. The tumblers were incubated at 20° C. for 10–12 days, after which the seedlings were washed and growth of *Ophiobolus* measured by Garrett's method (1936). Illustrative results are given in Table IV.

It is seen that *Ophiobolus* mycelium subjected for 5–7 days to contact with the staled media of certain fungi is rendered non-pathogenic. Similar preparations from other fungi have no effect, and those from yet others show intermediate behaviour. In a few cases (*Penicillium* D, E and G in Table IV) treatment of the *Ophiobolus* inoculum with the fungal preparations increased its pathogenicity.

Metabolic products of bacterial isolates were similarly tested after 15–20 days' growth: infection was suppressed in some cases, retarded in others.

Table IV. *The effect of preparations from 5-day-old cultures of various fungi upon pathogenicity of* Ophiobolus

Organisms	Growth of *Ophiobolus* along the root in cm. Col. I, staled media	Col. II, control	Col. I ÷ col. II %
Group I, showing no attack			
Aspergillus A	0·0	3·5	0·0
Penicillium C	0·0	2·3	0·0
,, F	0·0	2·2	0·0
,, H	0·0	2·8	0·0
Pythium	0·0	2·8	0·0
Trichoderma A	0·0	3·2	0·0
,, B	0·0	3·2	0·0
Group II, showing attack of less than 1 cm.			
Aspergillus C	0·9	2·8	31
Botrytis cinerea	0·7	2·5	28
Fusarium B	0·8	2·8	28
Penicillium I	0·9	2·8	30
Rhizoctonia	0·6	2·8	16
Unidentified A	0·9	2·8	32
,, B	0·9	2·8	31
Group III, showing an attack of 1 cm. or over			
Acrostalagmus cinnabarinus	1·3	2·5	52
Aspergillus B	2·0	2·3	87
Fusarium A	1·5	2·3	65
Penicillium A	2·0	2·5	80
,, D	2·9	2·2	131
,, E	3·3	2·2	148
,, G	2·9	2·2	127
Trichoderma C	1·8	3·2	56
Verticillium A	1·2	2·5	48
,, B	1·8	2·3	78

(4) *Soil organisms as affecting the pathogenicity of* Ophiobolus *in soil*

That soil organisms exercise a suppressive effect on *Ophiobolus* was shown by the following preliminary experiment. Presoaked wheat seeds were sown over *Ophiobolus* inoculum in sand which had been used repeatedly for growing wheat and oat seedlings. The sand used for control was sterilized by heat. The tumblers were incubated at 20° C. for 10 days. Examination of the seedling roots after washing showed that while the fungus had advanced 3·5 cm. along the roots in the sterilized sand there was hardly any initiation of attack on the roots of seedlings grown in oft-used sand. This suggests that the antibiotic activity of micro-organisms in the used sand is responsible for the effect.

A detailed experiment was set up to determine the inhibitive influence of individual organisms on the infective capacity of *Ophiobolus* in soil. The organisms employed were those used in the previous sections.

Ophiobolus and other organisms were developed on Slough soil adjusted to *p*H 7·0, containing 5% of cornmeal in Petri dishes, previous test having shown that such an inoculum of *Ophiobolus* is actively parasitic. Soil with 75% moisture, previously sterilized for three successive days at 10 lb. pressure for an hour, was distributed in sterile tumblers, roughly 200 g. being placed in each. The soil in each tumbler was gently pressed and over this was placed 5 g. of one of the following:

A. Sterilized soil and cornmeal mixture without any organisms.

B. Soil cornmeal mixture inoculated with *Ophiobolus* alone.

C. Equal quantities of *Ophiobolus* inoculum as in B and of the corresponding inoculum of a soil organism.

D. Soil cornmeal mixture inoculated with a soil organism.

Presoaked seeds of wheat treated with 1 : 320 formalin solution for ½ hr. were sown, five in each tumbler. The seeds and the inoculum were covered with sterilized soil, each treatment being replicated as a rule three times. The tumblers were incubated for the first 5 days at 20° C., after which they were removed to the open to allow of the normal growth of the emerging seedlings. Sterile water was sprinkled over the surface of the soil in the tumblers when necessary.

After the lapse of 2 weeks the heights of the plants were recorded, the roots then removed from the soil and washed and the percentage of primary roots discoloured by fungal attack determined. The percentage emergence of seedlings was noted. This varied from 75 to 93 % of seed sown, but as the variation showed no relation to the amount of root attack the figures are omitted. Illustrative data are given in Table V.

A similar range of results was given in experiments with the various bacterial isolates.

The following points may be noted in Table V:

(1) The height of the plants, when *Ophiobolus* inoculum alone is used, is considerably lower (2·7 in.) than in the uninoculated controls (6·2 in.).

(2) The height of the plants in presence of mixed inocula, although very variable (3·7–6·8 in.), is markedly greater than when *Ophiobolus* inoculum alone is used (2·7 in.).

(3) The height of the plants in presence of mixed *Ophiobolus* and contaminant inoculum is, in general, less than in presence of contaminant alone. This difference is most distinct the higher the percentage of root infection (Group III). Marked exceptions are seen in the case of *Penicillium* F and *Fusarium culmorum*. The latter is certainly a root parasite and the same is possible of the former.

Table V. *Effect of soil organisms upon pathogenicity of* Ophiobolus

Type of inoculum	Av. height (in.) of plants in presence of: Mixed inoculum	Av. height (in.) of plants in presence of: Single inoculum	% infection of primary roots
No organism	—	6·2	0
Ophiobolus alone	—	2·7	100
Fungal group I (infection <10%)			
Ophiobolus + *Fusarium* A	6·4	6·8	0
Ophiobolus + *Penicillium* C	6·0	6·3	9
Ophiobolus + *Pythium*	5·9	6·1	8
Ophiobolus + *Trichoderma* A	6·2	5·8	3
Ophiobolus + *Trichoderma* B	6·8	7·0	0
Ophiobolus + *Verticillium* B	6·3	6·8	5
Fungal group II (infection 10–30%)			
Ophiobolus + *Aspergillus* A	5·8	5·6	20
Ophiobolus + *Aspergillus* C	5·9	6·5	20
Ophiobolus + *Penicillium* B	5·6	6·5	27
Ophiobolus + *Penicillium* E	6·0	6·8	18
Ophiobolus + *Penicillium* F	6·6	3·9	30
Ophiobolus + *Penicillium* G	6·0	6·6	13
Ophiobolus + *Penicillium* I	6·1	6·4	15
Ophiobolus + *Rhizoctonia*	5·8	6·4	24
Fungal group III (infection >30%)			
Ophiobolus + *Acrostalagmus*	4·4	7·0	90
Ophiobolus + *Aspergillus* B	5·3	6·4	63
Ophiobolus + *Bot. cinerea*	3·7	5·8	100
Ophiobolus + *Fus. culmorum*	5·8	4·4*	50
Ophiobolus + *Penicillium* A	4·7	6·8	100
Ophiobolus + *Penicillium* D	4·6	5·8	81
Ophiobolus + *Penicillium* H	4·6	6·8	55

* Some browning of root.

(4) Certain organisms, viz. *Penicillium* F and H, which completely repressed *Ophiobolus* by their metabolic products in artificial medium (see Table IV), are not so effective in suppressing the activity of *Ophiobolus* in soil.

(5) The antibiotic effect of the various organisms on *Ophiobolus* is variable, ranging from nil to complete inhibition of its attack.

Summary

1. Isolations of *Ophiobolus* from infected wheat roots gave the maximum percentage of recovery 2–4 weeks after inoculation. Later the percentage of recovery declined. Disappearance of *Ophiobolus* mycelium was most rapid in acid soils, and slower in sand and in alkaline soil. In soils of more normal type an intermediate behaviour was shown.

2. From such infected roots a large number of soil fungi and bacteria were isolated and, in part, identified. It is suggested that disappearance of *Ophiobolus* mycelium from invaded roots is conditioned by the activity of these contaminants, and more particularly of such species as *Trichoderma lignorum*. Long persistence of the parasitic mycelium in roots growing in sand suggests that this is a poor medium for the growth of micro-organisms.

3. A systematic study in plate cultures of the interaction between *Ophiobolus* and the various soil contaminants isolated showed various degrees of interference with the growth of the former. These effects are described and tabulated. Similar interference effects were seen when *Ophiobolus* was grown in sterilized soil which had been inoculated with each of the contaminants.

4. These effects of the living contaminants can, to some extent, be reproduced by their metabolic products, viz. by filtered cultural solutions in which these organisms have grown. The inhibiting or retarding effects so produced are not due to the abstraction of food substances but to the presence of deleterious metabolites. In certain cases it was shown that *Ophiobolus* mycelium when exposed to such metabolic products was killed. This result was established both by cultural and pathogenic tests.

5. Tests with wheat seedlings planted over *Ophiobolus* mycelium, with or without the simultaneous presence of the various contaminating organisms, showed that the antibiotic effect varied from no appreciable effect to complete inhibition of attack. The various organisms are divided into three groups on this basis.

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STUDIES IN BACTERIOSIS. XXIV

STUDIES ON A BACTERIUM ASSOCIATED WITH LEAFY GALLS, FASCIATIONS AND "CAULIFLOWER" DISEASE OF VARIOUS PLANTS. PART III. FURTHER ISOLATIONS, INOCULATION EXPERIMENTS AND CULTURAL STUDIES[1]

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(With Plates XVII and XVIII)

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Introduction

In Part I of this series (Lacey, 1936 *a*) it was stated that Tilford, at the 26th and 27th Annual Meetings of the American Phytopathological Society, had reported the isolation of a pathogenic organism from fasciated sweet peas. Tilford (1936) proposed the name *Phytomonas fascians* for this organism, which, according to the description of its cultural characteristics, is identical with the species isolated by the writer from fasciations and leafy galls on various plants. As, however, by a resolution of the 2nd International Congress for Microbiology, 1936, the generic name "*Phytomonas*" for plant pathogenic bacteria is invalid, in the present paper the organism is referred to as *Bacterium fascians* (Migula's system). In addition to sweet peas, Tilford isolated *Bact. fascians* from growths at the base of chrysanthemums and a geranium and, by inoculation, produced fasciations on sweet peas, garden peas, petunia, tobacco, *Gypsophila paniculata* and geranium.

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THE ISOLATION OF *BACT. FASCIANS* FROM ABNORMAL GROWTHS ON VARIOUS HOST PLANTS

Part I of this series (Lacey, 1936 *a*) described the isolation of a pathogenic bacterium from fasciated sweet peas, leafy galls on chrysanthemums, carnations and *Schizanthus* and from "cauliflower" strawberries, and in Part II (Lacey, 1936 *b*) it was stated that isolations of the same organism had been made from galls on *Nicotiana glutinosa*, *Asparagus sprengeri*, *Heuchera sanguinea* and a gladiolus corm. Further details of these and of subsequent isolations are given below.

New isolations of *Bact. fascians* have been made from sweet pea, *Schizanthus*, carnation and chrysanthemum leafy galls, including two varieties of the Cascade chrysanthemum and a witches' broom type of growth on carnation.

The *Nicotiana glutinosa* plant from which the first isolation was made had a large gall surrounding the base of the stem similar in appearance to a chrysanthemum leafy gall. This plant was sent to the writer in March 1936 by Dr G. C. Ainsworth of the Experimental and Research Station, Cheshunt. In March 1937, Dr Ainsworth sent further specimens of *N. glutinosa* bearing very large leafy galls and also one plant of *N. tabacum* with a small leafy gall at the base of the stem. From both of these species typical cultures of *Bact. fascians* were isolated. Dr Ainsworth gives the following information:

"In connexion with the virus disease investigations here we raise several thousand *N. glutinosa* and *N. tabacum* each year and every year a few *N. glutinosa* and a smaller number of *N. tabacum* develop leafy galls at soil level. It has been noticed that the incidence of this disease of tobaccos is frequently correlated with the use of composts prepared from soil in which chrysanthemums (known to be slightly infected with "leafy gall") have been grown, although the soil is all sterilized by baking before use. Not one of the many thousand tomato plants grown at the same time and in the same composts as the tobaccos has ever developed a gall."

Asparagus sprengeri. In February 1936 Miss Walker of Swanley Horticultural College, Kent, sent fronds of *Asparagus sprengeri* bearing nodal galls on the aerial shoots which were similar in appearance to the type of stem gall produced by *Pseudomonas tumefaciens* (Pl. XVII, fig. 1). Some years previously the writer tried to isolate *Ps. tumefaciens* from similar galls on *Asparagus sprengeri* without success; *Bact. fascians* was easily isolated from these new specimens. Later, Miss Walker sent

two more plants of *Asparagus sprengeri* which, in addition to a few small aerial galls, had large masses of growth surrounding the base of the stems, partly above and partly below soil level. These basal galls were of considerably more compact and solid structure than the leafy galls of chrysanthemums, etc., but again numerous virulent colonies of *Bact. fascians* developed on all the isolation plates made from the growths. In June 1937 Dr Pape, of the Biological Station, Kiel, Germany, sent a basal stem gall of *Asparagus sprengeri*, similar to the Swanley specimens, from which a virulent culture of *Bact. fascians*, identical with the English strains, was isolated.

Brown & Weiss (1937) in America reported that they had failed to isolate *Pseudomonas tumefaciens* from fasciated galls on two specimens of *Asparagus sprengeri*, although the neoplasms were believed to be of the crown-gall type. It is probable that in this case, also, the causal agent was *Bact. fascians*.

"*Cauliflower*" *of* Heuchera sanguinea. These plants were sent to Dr T. Goodey, of the Institute of Agricultural Parasitology, by a grower who suspected eelworms as the cause of the disease. Dr Goodey found that, as in the case of "cauliflower" of strawberries, a species of eelworm, *Aphelenchoides fragariae* or *A. pseudolesistus*, was living ectoparasitically among the compact bases of the leaves, but he did not consider this to be the primary cause of the trouble and sent the plants to the writer, who isolated *Bact. fascians* from the growths. Dr Goodey sent further specimens of the same trouble a year later, and again numerous colonies of *Bact. fascians* were obtained on all the isolation plates.

The original gladiolus corm was sent by Mr Green, mycologist of the Royal Horticultural Society's Gardens, Wisley, and a second specimen was received later from Dr Ware of Wye Agricultural College. In both these specimens gall tissue had developed in place of the young cormels.

New host plants

Strains of *Bact. fascians* have been isolated from typical leafy galls on dahlia, pelargonium, petunia, crassula, hollyhock, nasturtium (Pl. XVII, fig. 2) and sweet William, and from abnormal growths on the following plants:

Shasta daisy (Chrysanthemum maximum). A group of Shasta daisy plants in a private garden had large leafy galls surrounding the bases of the stems and also fasciated growths, of a witches' broom type, in the axils of many of the lower leaves.

Cardamine triloba. This specimen bore numerous very short fasciated shoots at ground level. Prof. Weiss, the sender, stated that he had seen similar growths in former years on *Primula juliae* in the same garden.

Lilium regale. This plant had two normal buds below ground, but at the bottom node a gall had developed in place of a new bulb. The growth, which consisted of a solid mass of tissue resembling a corm in consistency and appearance, was loosely attached to the stem by a slender stalk.

Forsythia suspensa. Hard, brown, wart-like galls had developed at the nodes of young twigs of this plant in place of the axillary buds (Pl. XVII, fig. 3). Mr Fox Wilson, entomologist of the Royal Horticultural Society's Gardens, Wisley, who had previously examined this specimen, found that the galls were not of insect origin.

Phaseolus multiflorus. A runner bean, sent by Dr H. W. Miles, of Manchester University, had galls similar to those on *Forsythia suspensa* formed high up the stem in the bud portions.

Cheiranthus allionii *and larkspur*. The growths on these two specimens were of the witches' broom type, the main stems being of normal growth but with tufts of very finely divided leafy structures in the axils of the leaves in place of lateral shoots.

Finally, in addition to the asparagus gall mentioned previously, isolations of *Bact. fascians* have been made from leafy galls on pelargonium, petunia, *Aster frikarti*, *Verbascum densiflorum*, *Chrysanthemum maximum* and from an extremely fasciated basal shoot of *Chrysanthemum indicum* (Pl. XVII, fig. 4) sent by Dr Pape from Germany. These German strains of *Bact. fascians* were identical with the English strains.

The host range of plants affected by *Bact. fascians* is evidently very wide. Up to the present isolations of this organism have been made from growths on plant species belonging to sixteen different families, as follows: Monocotyledons-Liliaceae, *Asparagus sprengeri* and *Lilium regale*; Iridaceae, gladiolus. Dicotyledons-Caryophyllaceae, carnation and sweet William; Compositae, chrysanthemum sp., aster and dahlia; Crassulaceae, *Crassula*; Cruciferae, *Cardamine trifolia* and *Cheiranthus allionii*; Geraniaceae, pelargonium; Leguminoseae, sweet pea and *Phaseolus multiflorus*; Malvaceae, hollyhock; Oleaceae, *Forsythia*; Ranunculaceae, larkspur; Rosaceae, strawberry; Saxifragaceae, *Heuchera*; Scrophulariaceae, *Verbascum*; Solanaceae, *Nicotiana* sp., *Schizanthus* and petunia and Tropaeolaceae, nasturtium. In view of this wide distribution it is noteworthy that within the families, genera, species and even varieties differ widely in their susceptibility to attack. For example,

among the Solanaceae, petunias are very susceptible to "leafy gall" but tomatoes and potatoes appear to be immune. *Schizanthus retusus* is much more susceptible than other *Schizanthus* species. In the nursery from which the original "leafy-gall" specimens of *S. retusus* were obtained, 50% or more of plants of this species had been affected each year for a considerable period, but the disease had never been seen on other species grown in close proximity. After inoculation with *Bact. fascians*, 90% of *Schizanthus retusus* plants developed large leafy galls while only 40% of inoculated *S. grandiflora* produced small fasciated outgrowths. Similarly, both under natural conditions and by inoculation *Nicotiana glutinosa* was found to be more susceptible than *N. tabacum*.

Considerable differences in varietal susceptibility have been observed in the nurseries by chrysanthemum and carnation growers. For example, "Mason's Bronze" and "November Sun" chrysanthemums are very susceptible but "W. Turner" is resistant, while among the carnations the "Spectrum" varieties are most liable to develop the disease.

Inoculation experiments

In Part I of this series (Lacey, 1936 *a*) details were given of the successful inoculation of sweet pea seedlings with strains of *Bact. fascians* isolated from growths on sweet peas, chrysanthemums, carnations, *Schizanthus* and "cauliflower" strawberry plants. Numerous further inoculations of sweet peas and of several of the other host plants have been made with these strains and, also, with more recently isolated cultures.

(1) *Sweet pea inoculations*

A routine procedure has been established for the isolation and rapid identification of *Bact. fascians* from diseased tissue.

Isolation plates of bouillon agar medium are inoculated with macerated material suspended in sterile water, colonies resembling *Bact. fascians* are subcultured on to bouillon agar and these subcultures are inoculated on to germinated sweet pea seeds in sterile bottles containing wet sand. A strongly virulent colony will produce marked fasciation in 10–14 days, but with weaker strains a month or more may elapse before infection is evident. This difference in pathogenicity was very marked in isolations made from *Nicotiana glutinosa* and *N. tabacum* leafy galls. Six colonies from each isolation were inoculated on to sweet pea seedlings; two of the *N. glutinosa* colonies induced fasciation in 14 days, four in 18 days, and all six gave strongly positive results in

3 weeks. In contrast, none of the seedlings inoculated with the *N. tabacum* colonies showed any sign of infection until the twenty-second day, when two were feebly positive; a third colony produced slight fasciation after one month and the remaining three were avirulent. *N. tabacum* is more resistant to "leafy-gall" disease than *N. glutinosa*; both under natural conditions and as the result of inoculation fewer and smaller galls are produced on the former than on the latter. These facts suggest some correlation between the weaker virulence of the *N. tabacum* strain of *Bact. fascians* and the stronger resistance of the host plant.

In the majority of isolations from the various host plants only a very few of the colonies tested failed to produce fasciation of sweet pea seedlings, but in certain cases all the colonies tested were avirulent to sweet peas. Physiological study has failed to detect any cultural differences between the virulent and avirulent strains.

Tilford (1936) obtained both "rough" and "smooth" colony types of *Bact. fascians* from his original sweet pea isolations. The writer has obtained only "smooth" colonies in original isolations but "rough" colonies developed in some cases under certain cultural conditions, e.g. in cultures subjected to drying and to the action of weak antiseptics. These "rough" colonies, with "smooth" types from the same plates, were tested on sweet pea seedlings, but no difference in virulence was detectable, both types producing equally severe fasciation.

(2) *Inoculation of chrysanthemum cuttings*

In October 1935 chrysanthemum cuttings from six varieties known to be susceptible to "leafy gall" were planted in pots, the soil of which was watered with cultures of various strains of *Bact. fascians*. Table I gives the results of these inoculations.

Table I. *Inoculation of chrysanthemum cuttings with strains of* Bact. fascians

	Uninoculated control		Inoculated with chrysanthemum strain 356		Inoculated with sweet pea strain 16	
Chrysanthemum variety	No. inoculated	No. diseased	No. inoculated	No. diseased	No. inoculated	No. diseased
Mason's Bronze	3	0	2	2	3	3
Red Admiral	3	0	2	2	3	1
Edith Cavell	3	0	2	0	2	1
Edward Page	2	0	2	1	2	2
May Wallace	2	0	2	1	.	.
Favourite	3	0	2	2	.	.
Total no.	16	0	12	8	10	7

Table I (*cont.*)

Chrysanthemum variety	Inoculated with carnation strain 353		Inoculated with *Schizanthus* strain 355		Inoculated with strawberry strain 358	
	No. inoculated	No. diseased	No. inoculated	No. diseased	No. inoculated	No. diseased
Mason's Bronze	2	2	2	2	2	2
Red Admiral	2	2	2	1	3	3
Edith Cavell	2	1	.	.	.	.
Edward Page	2	0	3	2	3	3
May Wallace	2	0	3	1	.	.
Favourite	2	2	.	.	2	0
Total no.	12	7	10	6	10	8

Ten weeks after inoculation one "Mason's Bronze" cutting inoculated with the carnation strain had developed fasciated basal shoots and, a month later, infection was evident in several of the cuttings inoculated with the strawberry, sweet pea and chrysanthemum strains. The cuttings inoculated with the *Schizanthus* strain did not produce hypertrophied shoots until 6 months after inoculation, when the controls were still free from disease. A few new infections occurred among the inoculated plants until nine months after inoculation, by which time most of the earlier produced galls had rotted. All the inoculated "Mason's Bronze" cuttings developed "leafy gall", this variety being the most susceptible of those tested.

In a second test twenty chrysanthemum cuttings (var. "Debutante") taken from healthy plants were planted in pots of sterilized soil. Five cuttings were left uninoculated, five were infected by watering the soil with a culture of the carnation strain (353) and five each with two new strains—the Shasta daisy strain 386 and the *Lilium regale* strain 388. Six weeks later several of the inoculated cuttings had developed hypertrophied basal shoots and, at the end of 2 months, three out of the five infected with the carnation strain and all those infected with the daisy and *Lilium regale* strains were diseased. Pl. XVIII, fig. 1 is a photograph of one of these cuttings taken 3 months after inoculation; all five of the controls were then still free from disease and remained so until the experiment was concluded 2 months later.

Since these preliminary tests a number of further inoculations of chrysanthemum cuttings, chiefly of varieties "Mason's Bronze" and "Debutante", have been made, either to determine the virulence of newly isolated strains of *Bact. fascians* or in connexion with experiments to control "leafy gall". In all, of 156 cuttings inoculated with strains of *Bact. fascians*, 101 developed "leafy gall". With one exception the control

cuttings in these experiments remained free from disease. In this case the cuttings were taken from plants that had had "leafy gall" but had apparently recovered, and three out of eight of the uninoculated control cuttings developed "leafy gall" from 9 to 14 weeks after planting; all the inoculated cuttings were positive 4–8 weeks after inoculation. The shortest time that elapsed between inoculation and the appearance of hypertrophied shoots at soil level was 1 month, when the cuttings were taken in the early spring (as in the usual nursery procedure). Cuttings taken in the autumn or late spring developed the disease more slowly and in fewer numbers.

In one nursery where "leafy gall" was rife the variety "W. Turner" was entirely unaffected. Nine cuttings of this variety were inoculated with *Bact. fascians* with negative results, thus confirming the field observation that "W. Turner" is markedly resistant to "leafy gall".

(3) *Inoculation of carnation cuttings*

Carnations are not so susceptible to "leafy gall" as chrysanthemums. In one nursery, where the disease has been present for 10–12 years, the grower stated that only about 5% of the carnations were seriously affected each year, the "Spectrum" variety being especially liable to attack.

In October 1935 forty carnation cuttings (variety "Salmon's Spectrum") were infected by watering the soil round the cuttings with *Bact. fascians* strains from sweet pea, carnation, chrysanthemum, *Schizanthus* and strawberry, eight cuttings being inoculated with each strain and another eight left uninoculated as controls. No definite sign of disease was apparent until May 1936 when a typical moss-like leafy gall appeared just above soil level on one cutting inoculated with the carnation strain. The gall was loosely attached to the stem by a thin stalk arising from a node 1 in. above the base of the cutting. Two of the cuttings inoculated with the *Schizanthus* strain showed similar, but smaller, growths. No further results could be obtained as, about this date, the plants were so badly damaged by cockroaches that the majority died. In a repeat experiment the following October twenty cuttings of "Salmon's Spectrum" were planted in sterilized soil. Sixteen of these were inoculated with *Bact. fascians* strains from carnation, sweet pea, *Schizanthus* or Shasta daisy and four were left uninoculated. In January three out of four of the cuttings inoculated with the sweet pea strain and one of those inoculated with the Shasta daisy strain had developed "leafy gall" and, later, one cutting inoculated with the carnation strain was

affected (Pl. XVIII, fig. 2). The controls and the cuttings inoculated with the *Schizanthus* strain remained free from disease. In a third experiment two out of four cuttings inoculated with a recently isolated chrysanthemum strain of *Bact. fascians* developed "leafy gall".

Although the number of positive infections (ten out of sixty) was low, the incidence of "leafy gall" among carnations inoculated with *Bact. fascians* was three times as great as that observed in the nursery.

(4) *Inoculation of* Schizanthus

(*a*) *Schizanthus retusus.* Of twenty young plants of *S. retusus* grown in sterilized soil and inoculated by pricking the stems at soil level with needles smeared with cultures of *Bact. fascians*, eighteen developed "leafy gall", infection being apparent in several of the plants 3 weeks after inoculation. In other experiments cultures were watered into the soil round young *Schizanthus retusus* plants, which were not wounded. Fourteen out of twenty-two of these plants developed "leafy gall". The incubation period was slightly longer and the percentage of positive infections somewhat lower than in plants treated by prick inoculations, but the presence of wounds is evidently not essential for the infection of *S. retusus* by *Bact. fascians.* Strains of *Bact. fascians* isolated from *Schizanthus*, sweet pea, chrysanthemum, strawberry and *Nicotiana glutinosa* were equally virulent to *Schizanthus retusus.*

(*b*) *Inoculation of* Schizanthus penatus *varieties.* Twenty-five young plants of *S. grandiflora* hybrids, grown in sterilized soil, were inoculated with *Schizanthus* strains of *Bact. fascians* by needle pricks at soil level. After 1 month three plants showed infection and later ten out of the twenty-five developed small leafy galls. The remaining fifteen and all the controls were negative. Two plants of *Schizanthus penatus*, grown in the nursery from which the original specimens of "leafy gall" *S. retusus* were obtained, were inoculated by needle pricks. One developed a leafy gall, the other was unaffected. Galls had never been seen on *S. penatus* plants grown in the nursery, where a large percentage of the *S. retusus* plants were affected each year, but it is evident that *S. penatus* is not entirely immune to the disease.

(5) *Inoculation of* Nicotiana *sp.*

Twenty-six young plants of *Nicotiana glutinosa* were inoculated with nine different strains of *Bact. fascians* by pricking the stems at soil level with needles smeared with culture. Twenty-five developed large leafy galls, the first signs being apparent eighteen days after inoculation.

Most of the plants were so seriously affected that they were greatly stunted, with very small, malformed leaves (Pl. XVIII, fig. 3). Six control plants developed normally. In a second experiment cultures of *Bact. fascians* were watered into the soil around eleven *Nicotiana glutinosa* plants, which were not wounded. All developed leafy galls as rapidly as those inoculated by needle pricks, thus proving that wounds are unnecessary for infection to take place.

Nicotiana tabacum. Of twenty-eight *N. tabacum* plants inoculated by needle pricks twenty-four developed leafy galls, but these remained small and did not affect the normal growth of the plant. Only one of three plants infected by watering the soil without wounding produced a very small gall. These results conform with the incidence of leafy galls under natural conditions, where, as previously noted, galls are produced much more freely and are of greater size on *N. glutinosa* than on *N. tabacum*.

(6) *Inoculation of* Heuchera sanguinea

Three young plants of *H. sanguinea* were inoculated with a *Bact. fascians* strain isolated from a *Heuchera* gall. Seventeen days later one of the plants showed definite signs of disease, the new leaves being small and deformed. After 2 months the entire crown presented a "cauliflower" appearance with tiny distorted leaves arising from a mass of galled tissue (Pl. XVIII, fig. 4). The other two inoculated plants developed a "leafy-gall" type of growth at the side of the crown, which was not itself affected. A control plant developed normally.

(7) *Inoculation of petunia*

Three inoculated petunia plants all developed large leafy galls at soil level, the growth being apparent 16–23 days after inoculation.

(8) *Inoculation of* Asparagus sprengeri

During April and June twelve seedlings of *A. sprengeri* were infected by watering the soil round the base of the plants with strains of *Bact. fascians* isolated from *Asparagus sprengeri*, chrysanthemum, petunia or sweet pea. No abnormal growth developed until October, by which time three of the plants had produced typical galls at soil level and during November three more plants became diseased.

(9) *Inoculation of* Pisum sativum, Phaseolus vulgaris *and* Vicia Faba

Strains of *Bact. fascians* from sweet pea, carnation, Shasta daisy or *Lilium regale* were watered into the soil and over the apical buds of thirty-five pea seedlings. Three out of nine of the plants infected with

the carnation strain and one infected with the Shasta daisy strain developed fasciated basal shoots similar to those produced on infected sweet peas; the rest gave negative results.

Twenty French bean seedlings were inoculated with various strains of *Bact. fascians* by placing drops of water infected with the culture on the growing point between the apical leaves and also in the axils of the first pair of leaves. With two exceptions, all the plants developed galls at the apical point and in the axils of the leaves, and normal growth was completely checked. Leaves which opened after the inoculation were distorted and mottled, with raised dark green patches interposed among sunken yellowish-green areas, giving a mosaic appearance. Inoculations made by infecting the soil at the time of planting were not so successful, for out of twenty-eight plants only one was seriously affected. This developed several fasciated shoots which ceased growth when not more than 3 in. high and a definite gall was produced at soil level. Twenty of thirty-five broad bean plants, growing in soil which was infected with *Bact. fascians* at the time of planting the seeds, developed hypertrophied basal shoots of the "leafy-gall" type.

Negative inoculations. Inoculation of tomato plants by soil infection, stem pricks and by smearing the apical and axillary buds with cultures of *Bact. fascians* all gave negative results. Typical galls were produced on the stems of tomato plants by prick inoculations with *Pseudomonas tumefaciens* under the same conditions. Inoculation of potato tubers also gave negative results.

Thirty gladiolus corms were inoculated 1 month after planting by pricking the organisms into the young shoots and also by infecting the soil. No abnormal growths developed. The galls on the original gladiolus specimens had developed from the buds which normally produce the little cormels, the main corm being unaffected (Lacey, 1936*b*, Pl. XXXIII, fig. 2). These cormels are formed rather late in the season and a probable explanation of the negative results is that the inoculation was made too early. The soil conditions were evidently unfavourable for the survival of *Bact. fascians*, since attempts to reisolate the pathogen from the exterior of the new corms and from the soil surrounding them, failed.

The inoculation of strawberry plants for the production of the "cauliflower" disease will be dealt with in a later paper.

A summary of the inoculation experiments is given in Table II.

Strains isolated from various host plants have been tested in these inoculation experiments to determine if there was any biological variation in pathogenicity among the strains. Four strains which were non-

Table II. *Summary of inoculation experiments with* Bact. fascians

Plant inoculated	Method of inoculation	No. inoculated	No. positive	% positive
Chrysanthemum:				
(*a*) Susceptible varieties	Cuttings in infected soil	156	101	65
(*b*) Resistant "W. Turner"	Cuttings in infected soil	9	0	0
Carnation	Cuttings in infected soil	60	10	16·6
Schizanthus:				
(*a*) *S. retusus*	(1) Prick inoculation	20	18	90
	(2) Infected soil	22	14	64
(*b*) *S. penatus*	Prick inoculation	27	11	41
Nicotiana:				
(*a*) *N. glutinosa*	(1) Prick inoculation	26	25	96
	(2) Infected soil	11	11	100
(*b*) *N. tabacum*	(1) Prick inoculation	28	24	86
	(2) Infected soil	3	1	33
Heuchera sanguinea	Prick inoculation	3	3	100
Petunia	Prick inoculation and soil infection	3	3	100
Asparagus sprengeri	Soil infection	12	6	50
Pisum sativum	Soil infection	35	4	11
Phaseolus vulgaris	(1) Inoculation of growing point	20	18	90
	(2) Soil infection	28	1	3·5
Vicia Faba	Soil infection	35	20	57
Gladiolus	Prick and soil infection	30	0	0

pathogenic to sweet peas were also avirulent to *Schizanthus retusus*, but eight strains virulent to sweet peas all produced leafy galls on *S. retusus*. Twelve strains pathogenic to sweet peas were also pathogenic to chrysanthemum cuttings, though there was some variation in the degree of infection, the length of the incubation period and the number of cuttings affected. Nine strains were tested on *Nicotiana glutinosa* and *N. tabacum*. All nine were pathogenic for both species and in every case the disease was more serious on *N. glutinosa* than on *N. tabacum*. Negative results were, however, obtained by the inoculation of *Pisum sativum* with the sweet pea and *Lilium regale* strains, and of *Vicia Faba* with the *Lilium regale* and Shasta daisy strains though these were virulent to sweet peas and chrysanthemums. The sweet pea, carnation, tobacco and chrysanthemum strains were all pathogenic to *Vicia Faba* and *Phaseolus vulgaris*.

These results show that the various strains of *Bact. fascians* have a wide host range. With the exception of the sweet pea and *Schizanthus* inoculations with avirulent strains, the number of plants inoculated in experiments in which a particular strain has failed to produce galls on any host has been too small to constitute a proof of complete avirulence to that host; further inoculations are necessary.

The examination of plants either naturally or artificially infected with *Bact. fascians* shows that the galls are invariably produced by the stimulation and abnormal proliferation of bud tissue. The plumules of sweet pea seedlings inoculated immediately after germination may become hypertrophied, but the greater part of the galled tissue develops from the lateral buds which in normal seedlings remain latent unless the plumule is injured. In plants such as *Nicotiana*, *Schizanthus*, etc., the leafy galls have their origin in the nodal buds at the base of the stem, which in normal plants do not develop. Chrysanthemums normally produce basal shoots from these buds and infection by *Bact. fascians* causes "leafy-gall" tissue to be formed in their stead. Buds in the axils of leaves of aerial stems may give rise to galls (*Phaseolus* sp., *Forsythia*) or the entire growing point may be involved, giving rise to the growths known as "cauliflower" (*Heuchera*, strawberry). Finally, as in gladiolus and *Lilium regale*, buds which normally develop into storage organs may be affected.

Attempts to produce abnormal proliferation of any tissues other than buds have failed. Prick inoculation of *Bact. fascians* into the internodes of young sweet pea seedlings have invariably been negative and no excess callus growth resulted from the inoculation of carrot slices kept in moist Petri dishes (a successful method of testing the virulence of *Pseudomonas tumefaciens*). Wounds are not necessary for gall formation by *Bact. fascians* but *Pseudomonas tumefaciens* has no action on an uninjured surface. Chrysanthemum cuttings may be attacked by both *Bact. fascians* and *Pseudomonas tumefaciens*, but whereas the former will produce a "leafy-gall" type of growth from the underground buds and have no effect on the cut end, the latter will cause the formation of a compact gall of undifferentiated tissue at the base of the cutting. A prick with a needle smeared with a culture of *Ps. tumefaciens* passing through the cambium in any part of an actively growing stem (e.g. tomato) will cause the formation of a wart-like gall; galls of similar appearance may be produced by infection with *Bact. fascians* (e.g. *Asparagus sprengeri*), but only at the nodes. *Bact. fascians* has no direct action on root tissue, and the roots of an infected plant are not affected unless the plant is greatly weakened by the disease.

Microscopical examinations of sections of young galls of *Schizanthus*, *Nicotiana* and chrysanthemum showed that, as in the sweet pea, the bacteria are mainly confined to the exterior of the growths. In some places a thick bacterial zoogloea was pressed against the outer walls of the epidermis, with occasional penetration into the intercellular spaces

and the formation of pockets of necrosed tissue due to the destruction of the epidermal and subepidermal cells. In the vicinity of the bacterial zoogloea active cellular division of the surrounding tissue caused the production of irregular protuberances in precisely the same manner as that seen to occur in inoculated sweet pea seedlings.

EVIDENCE THAT *BACT. FASCIANS* MAY BE SEED-BORNE

Tilford (1936) states that *Bact. fascians* may be carried on the exterior of the seed coat of sweet peas. The history of "leafy gall" on *Schizanthus retusus* suggested that, in this plant also, the disease might be seed-borne. In the nursery from which the original specimens were obtained the disease had appeared every spring for a number of years on *S. retusus* plants, of which more than one-half were invariably infected. The disease usually developed rapidly after the plants had been potted when about 6 in. high. Soil sterilization had failed to check the disease and, as the potting soil was imported fresh each year from various parts and no variation in the amount of disease was noticeable from year to year, the soil would seem to be eliminated as a source of infection. The seed, however, was collected indiscriminately from both healthy plants and those not too seriously affected by the disease to produce normal flowers.

In the spring of 1936 seeds of the 1935 crop of *S. retusus* from the infected nursery were planted in pots of sterilized soil. Fifty-four seeds germinated and 2 months after planting one of these showed definite signs of disease, the hypocotyl being greatly swollen and the first leaves small and yellow. Numerous colonies of *Bact. fascians* were obtained on isolation plates made from the hypocotyl tissue. Later, another seedling developed a leafy gall from which *Bact. fascians* was isolated.

In a second experiment a number of seeds of the 1936 crop of *Schizanthus retusus* from the infected nursery were embedded in bouillon agar. The seeds became covered with a mixed bacterial growth, amongst which, in several cases, were organisms resembling *Bact. fascians*. The mixed growth from twelve of the seeds was inoculated on to sweet pea seedlings, two of which developed fasciations from which *Bact. fascians* was isolated. The percentage of infected seeds in these samples was evidently small, but with even one diseased seedling in a seed box the infection might become widespread through watering or by the contamination of the hands or tools during transplanting.

Physiological tests

A study of the physiological reactions of forty strains of *Bact. fascians* has failed to disclose any means by which avirulent and virulent strains can be distinguished in culture. The work has, however, yielded some interesting results. It was stated in Part I of this series (Lacey, 1936 *a*) that while the production of acid from sugars in synthetic media was very slow and feeble (being determined by the change in colour from blue to yellow of brom-thymol-blue indicator in the medium), yet this afforded some means of differentiation between the strains, in that the carnation, chrysanthemum and strawberry strains produced acid from lactose and the *Schizanthus* and sweet pea strains did not. By a continuation of these tests the various strains of *Bact. fascians* were divided into two main groups—those that produced acid from lactose and non-lactose fermenters. These were each separated into two sub-groups (*a*) producing slight acid from mannitol, (*b*) causing no increase in the hydrogen-ion concentration of mannitol media. All the strains produced some acid from glucose and saccharose in varying degrees. Virulent and avirulent strains occurred in each group but in most cases the strains isolated from a particular host plant were identical in cultural reactions. For example, the *Asparagus sprengeri* strain from Germany and the two English strains were in close agreement not only in failing to produce acid from lactose but also in the amount of the increase of the hydrogen-ion concentration from day to day of the glucose, saccharose and mannitol cultures. Similarly, a strain isolated from a pelargonium gall from Germany was identical with the English strains from the same host. The strawberry and *Heuchera* strains, all of which were isolated from "cauliflower" growths, were in group II *b* (acid from lactose, but not from mannitol) and a sweet pea strain, isolated three years after the original isolations were made, was identical with the latter. An exception was found in the chrysanthemum strains, for of the six cultures tested, three produced acid from lactose and three did not; all six were virulent on sweet pea.

Eight strains (sweet pea, asparagus, *Schizanthus*, pelargonium, carnation, chrysanthemum, dahlia and strawberry) were tested in a number of other carbohydrate synthetic media. All eight produced acid from levulose, galactose and mannose, and slightly increased the hydrogen-ion concentration in the xylose and maltose tubes, but had no action on dulcite, dextrin, sorbitol and inulin. The first six strains had a feeble action on arabinose, while the dahlia and strawberry strains were negative, and the pelargonium strain alone produced acid from rhamnose.

Liquefaction of gelatine

In the first paper of this series (Lacey, 1936 *a*) and according to Tilford (1936), *Bact. fascians* was found to be a non-liquefier of gelatine at 20° C. Further tests have shown that, when the cultures were incubated at 24° C., liquefaction began at the surface of a stab culture in 2–4 weeks and the medium gradually became of a thick treacly consistency. This change did not occur in gelatine stabs at 24° C. inoculated with, e.g., *Pseudomonas fluorescens non-liquefaciens*. (A similar type of liquefaction was observed by Burkholder (1930) to occur in gelatine cultures of *Ps. medicaginis* var. *phaseolicola*.) The organism cannot be said to be a true gelatine-liquefier, but the reaction is characteristic of this species.

Litmus milk. All the strains of *Bact. fascians* produced a deep blue coloration in litmus milk. In the majority of cultures a soft curd was slowly formed and still more slowly digested, but these reactions varied with the different batches of milk used and there was no uniformity among strains isolated from any one host plant.

Nitrate reduction. In the original description, this organism was stated to be a non-nitrate reducer, the tests having been made on nitrate broth and on liquid synthetic nitrate media. Tilford (1936), however, obtained feeble nitrate reduction on synthetic nitrate agar, but not on peptone beef-extract agar. Further tests of the strains of *Bact. fascians* isolated by the writer have confirmed Tilford's results, in that most of the strains will feebly reduce nitrate when grown on synthetic nitrate agar, but not on fluid synthetic nitrate or peptone media.

SUMMARY

1. *Bact. fascians* (Tilford), the causal agent of fasciation of sweet peas, leafy galls, etc., has been isolated from abnormal growths on plants of twenty-five different genera belonging to sixteen families, including both Monocotyledons and Dicotyledons.

2. All these strains of *Bact. fascians* were inoculated on to sweet pea seedlings which, in the majority of cases, became severely fasciated.

3. Characteristic galls have been produced on chrysanthemums, carnations, *Schizanthus* sp., *Nicotiana* sp., *Heuchera sanguinea*, petunia, *Asparagus sprengeri*, *Pisum sativum*, *Phaseolus vulgaris* and *Vicia Faba* by inoculation with various strains of *Bact. fascians*. No distinct biological variations among the strains could be detected.

4. Inoculation of gladiolus, tomato and potato gave negative results.

5. The galls are invariably produced in the region of bud tissue. In young growths the bacteria are mainly confined to the exterior of the galls.

6. The organism may be carried on the seeds of *Schizanthus*.

7. A study of the physiological reactions of forty strains of *Bact. fascians* has failed to disclose any means by which virulent and avirulent strains can be distinguished in culture.

The author takes this opportunity to express her gratitude to the senders of the numerous specimens examined during the course of this work. In particular she wishes to thank Prof. W. Brown, of the Imperial College of Science, and Mr W. Buddin, Advisory Mycologist of the Ministry of Agriculture, both for their help in procuring material and for their interest and advice.

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EXPLANATION OF PLATES XVII AND XVIII

Plate XVII

Fig. 1. Galls on *Asparagus sprengeri* fronds. Nat. infection.

Fig. 2. "Fasciation" of nasturtium. Nat. infection.

Fig. 3. Galls on *Forsythia suspensa*. Nat. infection.

Fig. 4. Fasciated shoot of *Chrysanthemum indicum*. Nat. infection.

Plate XVIII

Fig. 1. Chrysanthemum cutting 3 months after infection with *Bact. fascians* (*Lilium regale* strain).

Fig. 2. Carnation cutting 6 months after infection with *Bact. fascians* (carnation strain).

Fig. 3. *Nicotiana glutinosa* seedling 6 weeks after infection with *Bact. fascians* (sweet pea strain).

Fig. 4. *Heuchera sanguinea* infected with *Bact. fascians* (*Heuchera* strain). Photographed 4 months after inoculation.

(*Received* 15 *December* 1938)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

LETTUCE MOSAIC

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(With Plates XIX and XX)

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Introduction

Mosaic disease of lettuce was first described in Florida, U.S.A., by Jagger (1921) and later by Brandenburg (1928) in Germany and by Ogilvie (1928) in Bermuda. Ogilvie & Mulligan (1931) were the first definitely to record the disease in England, where mosaic has since been noted by Pethybridge *et al.* (1934), and, on lettuce under glass, by Ainsworth (1937). It is probable that all these investigators studied the same disease but the causal virus has never been described, and the purpose of this paper is to place on record a description of the virus and a more detailed account of the disease than has hitherto been published.

Methods

Most of the field observations were made in the west of England, particularly in the Evesham market garden area and on experimental plantings at Long Ashton, while the work involving experimental

inoculations was carried out under controlled conditions under glass at Cheshunt.

Lettuce plants for inoculation experiments were raised from virus-free seed in an insect-free glasshouse, and for making routine tests seedlings growing in 3 in. pots were used. The variety Cheshunt Early Giant, a cabbage lettuce of the Gotte group, was employed almost exclusively as the test plant.

The method of inoculation finally developed was to grind infected leaves with a little 0·5% sodium sulphite solution and, after the addition of a small quantity of powdered carborundum, inoculate leaves by gentle rubbing with a pad of butter muslin moistened with inoculum, when 80–100% infection of lettuce resulted.

For the experiments on insect transmission suitable insect-proof cages were employed.

Symptoms

The symptoms on mature cabbage lettuce (*Lactuca sativa* L. var. *capitata* Hort.) in the field, at the time when normal plants are ready for cutting, comprise dwarfing, defective hearting, and mottling or yellowing, necrosis or scorching, and distortion of the leaves. The mottling and yellowing symptoms are most noticeable during the spring and necrosis and scorching during the summer. Clearing of the veins is a common symptom in both young and old plants, and in addition there may be an irregular pale blotching, or the whole leaf may simply be rather yellow and hard looking, as if injured by wind or frost. The leaves may also be more blistered or ballooned than usual and have their shape further altered by emphasis of the serrations so that the characteristic features of a variety may become disguised. Necrosis takes the form of numerous minute necrotic brown spots between the veins or a more definite vein necrosis, and severe scorching of the leaf edges may occur. The effect of mosaic is particularly noticeable when the plants "bolt". A diseased plant is stunted, the leaves on stem and inflorescences show a pronounced mottle and sometimes necrosis, while in certain varieties necrotic lesions develop on the stem, the bracts, and the flower buds (Pl. XX, fig. 2 and cf. Brandenburg (1928), p. 45, Abb. 11), when flowers may fail to open. The amount of seed is reduced. In general, diseased flowering plants may be distinguished by their yellow colour, scorched appearance and shortness.

Under experimental conditions in the glasshouse the first symptom, which appears 7–14 (usually about 10) days after inoculation by either

mechanical means or insects, is a pronounced clearing of the veins of the younger leaves (Pl. XIX, fig. 2). Later, the vein-clearing becomes either less pronounced, when a more general mottling of the leaves is seen, or more pronounced so that a definite necrosis of the veins occurs (Pl. XIX, fig. 1). Affected plants are usually paler in colour than corresponding healthy plants, satisfactory hearting may be interfered with, and there is a greater or less degree of stunting (Pl. XX, fig. 1). Most of the symptoms observed in the field have been reproduced experimentally, but an important factor for symptom expression in cabbage lettuces is the variety infected. This is dealt with in the succeeding section.

Inoculations made under glass at different times of the year have indicated little seasonal effect on either the incubation period or the general symptoms, but, in the case of Cheshunt Early Giant, the variety used most extensively, vein necrosis tended to be replaced in mid-winter by severe yellowing of the veins. The severity of the symptoms shown by an individual plant is to some extent correlated with its size when inoculated; a seedling plant being more badly stunted, etc., than a plant inoculated at a later stage of development, and this effect is also evident in the field.

In cos lettuce (*L. sativa* L. var. *romana* Hort.) the first symptom is a clearing of the veins, and subsequently a more or less pronounced mottle develops (Pl. XX, fig. 4). Vein necrosis is rare, but scorching of the leaf edges tends to occur and the plants are stunted (see Ogilvie *et al.* 1935, p. 183, plate 2, fig. 2). Infected plants fail to make a compact heart (Pl. XX, fig. 3), and this symptom is useful in distinguishing diseased and healthy plants when the mottling is rather indefinite. Several varieties of cos lettuce have been inoculated in the glasshouse but there has been little variation in the symptoms and a similar uniformity has been observed in the field.

Although mosaic diseased plants can usually be recognized without difficulty in the field, the differences in varietal reaction combined with varying environmental conditions and the effects of aphis infestation, unfavourable soil conditions and root rots may, especially on outdoor winter lettuce, make the certain diagnosis of mosaic impossible without recourse to inoculation tests.

Varietal reactions of cabbage lettuces to mosaic

It was evident from the observations of Ogilvie & Brian (1936) that there were considerable differences between the reactions of different

varieties of cabbage lettuce to mosaic. In order to examine this point twenty-two different varieties of cabbage lettuce were raised from seed under the same conditions in a heated glasshouse, and about five plants of each variety were inoculated at the five-leaf stage and, subsequently, five more plants of each variety at the eight- to nine-leaf stage. The plants were growing in 3 in. pots when inoculated but, later, infected and healthy plants of both series were transferred to 6 in. pots and kept under observation for approximately 2 months (March–May) after inoculation. These tests are summarized in Table I. When the lettuce varieties employed were grouped according to Brian's (1936) classification it was found possible to make certain generalizations concerning the symptoms exhibited by the members of the different groups. With a few exceptions groups 2, 3, 4, 6 and 8 were characterized by mottle symptoms while in groups 7, 5 and 1 the necrotic type of symptom was severe. (In the notes which accompany Table I the reactions of lettuces of the different groups when inoculated under experimental conditions are set out parallel with the observations made on the behaviour of the same types in the field.)

Little evidence was found to indicate any differences in susceptibility between any of the varieties tested, but the symptoms ranged from the severely necrotic type exhibited by Whitsuntide (Pl. XIX, fig. 1) to the mild mottling exhibited by members of the Trocadero and Crisp (Pl. XIX, fig. 3) groups.

It will be seen that while the inoculation tests have shown large differences in the varietal reaction of cabbage lettuces to mosaic, various discrepancies exist between the estimate of the general severity of the disease under experimental conditions and the estimate for field conditions.

It must be remembered that most of the lettuces inoculated experimentally were types not intended to be forced under glass (see the last column of Table I) and that the estimate of disease intensity in the field has a commercial bias. For example, a variety mildly attacked as judged by the general symptoms would be classed as severely affected in the field if unsatisfactory hearting resulted when the lettuce was grown at its appropriate season.

(1) *Gotte*

Experimental. Moderately severe mottle, moderate to severe vein necrosis, and rather severe stunting were typical of this group (Pl. XX, fig. 1). Resistant Early French Frame was less badly affected than the other two varieties of the Gotte group tested, the mottle was slight and there was very little necrosis, and this variety and

Table I. *Reaction of cabbage lettuces to mosaic*

Group (Brian's classification)	Variety	% infection*	Symptoms	General reaction: Experimental inoculation	General reaction: Field estimate	Type of lettuce
(1) Gotte	Resistant Early French Frame	20	m, n (trace), ms, S	Severe	—	F
	Tennis Ball B.S.	40	M, n, S	Very severe	Severe	F
	Cheshunt Early Giant†	80	M, n–N, ms, S	Severe	—	F
(2) All the Year Round	All the Year Round	90	m, n (trace), s	Severe	Severe	Sp, S (W)
	Feltham King	80	M, n (trace), s	Severe	Severe	Sp, S (W) (F)
	Spring Beauty	100	M, s	Severe	Severe	Sp, S, W
(3) May Queen	May King	100	M, s	Severe	Severe	F, Sp
(4) Trocadero	Trocadero	90	m, s (slight)	Mild	Mild	Sp, W
	Improved Trocadero	78	M, n (trace), s	Severe	Severe	S, W
	Premium	90	m–M, n (trace), s	Mild	Mild	S, W
	Unrivalled	89	M, n (trace), s	Mild	Severe	Sp, S, W
	White Boston	78	m, n (trace), s	Mild	—	Sp, S, W
(5) Lee's Immense	Lee's Immense	100	M, n–N, ms, S	Very severe	Mild	W
	Harrison's Green Winter	80	m, n, MS, s	Severe	Mild	W
	Yates's Winter White	60	M, n (trace), s	Severe	Severe	W
(6) Passion	Arctic (Sutton)	90	M, ms, s	Severe	Severe	W
	Arctic (Hurst)	90	M, s	Severe	Severe	W
	Arctic King	100	M, s	Severe	Severe	W
	Imperial	100	M, n–N, ms, S	Very severe	Severe	W
	Stanstead Park	100	m–M, s	Mild	Mild	W
(7) MacHattie's Giant	Whitsuntide	90	M, N, S	Very severe	Severe	W
(8) Crisp	Iceberg	100	m–M, s (slight)	Mild	Severe	S
	Webb's Wonderful	77	m–M, s (slight)	Mild	Severe	S

Symptoms: M, Vein-clearing, mottle
N, Vein necrosis
MS, Marginal scorch
S, Stunting

Lettuce type: Sp, Spring
S, Summer
W, Winter
F, Forcing

(Capitals, symptoms intense; small letters, symptoms less well developed.)

* Average $\frac{188}{226} = 83{\cdot}5\,\%$.

† Cheshunt Early Ball reacts in a similar manner.

Tennis Ball B.S. showed the two lowest percentage infections of the whole series. The low percentage infections may perhaps be accounted for by the rather leathery leaf texture shown by these varieties, which makes artificial inoculation more difficult. Once infected, however, plants of these two varieties showed little tolerance to the virus.

Field observations. In the Gotte class symptoms are severe. The plants are greatly dwarfed and mottled or yellow. Necrosis is not very marked. The leaves of the flower stalks are greatly reduced in size.

(2) *All the Year Round*

Experimental. A mild to moderately severe mottle was a predominating symptom of this group.

Field observations. In the pale varieties of the All the Year Round group the symptoms are severe. If infected at an early stage the size of the plant is considerably reduced. The leaves are yellowed or mottled with dark areas near the veins and the rest of the leaf pale, more blistered than usual and with necrotic spots between the veins. There may be marked scorching of the leaf edges in the summer. The leaf serration may be greatly emphasized.

In the dark green All the Year Round varieties the symptoms are slight, only slight hardening of the outer surfaces of the leaves and slight scorching and slight failure to heart.

In the tinted All the Year Round varieties the leaves assume a brownish colour.

In Spring Beauty and Feltham King the plants are so slightly affected as usually to be saleable.

(3) *May Queen*

Experimental. May King, which was the only representative of the group tested, showed a very pronounced yellowing of the veins as the early symptoms (Pl. XIX, fig. 2) and, later, a general mottle and stunting, but no necrosis.

Field observations. In the May Queen group the leaves are conspicuously mottled or yellow in colour, the plants are stunted and hearting is poor.

(4) *Trocadero*

Experimental. Lettuces of this group proved rather tolerant to the virus. Trocadero showed a mild mottle without necrosis and slight stunting while Improved Trocadero was rather more severely attacked. The remaining varieties were intermediate between these two types.

Field observations. In the Trocadero group there is marked blistering of the leaf. An accentuation of the serration of the leaf edges alters the appearance of the lettuce considerably. The affected plants are paler than normal, but hearting is usually little affected.

(5) *Lee's Immense*

Experimental. Mottle, necrosis and stunting were all pronounced. Necrosis was most severe in Lee's Immense and least severe in Yates's Winter White. Harrison's Green Winter showed particularly severe marginal scorch.

Field observations. In the Lee's Immense group the plants are usually small, pale and slightly mottled, while hearting may be only slightly or considerably reduced.

(6) *Passion*

Experimental. As a group these varieties were characterized by a well-developed mottle, or very slight necrosis, and slight stunting. Imperial was an exception and showed rather severe necrosis and more pronounced stunting.

Field observations. In the Arctic types the plants are greatly dwarfed. The leaves are pale or mottled and much crinkled. There is little or no hearting.

On the flowering stem of Passion varieties grown in summer numerous elongated brown necrotic spots may occur. These coalesce and lead to a general browning or to elongated lesions which eventually may split with exudation of latex. There is also a necrosis of the small leaves of the flowering stem.

In the Stanstead Park types there is only a slight paling of the leaves, with little reduction in hearting. Normally the leaves are smooth, but in mosaic-infected plants they are often markedly corrugated. The leaflets on the flower stems show clearing of the veins and are irregularly blotched, the pale areas being mostly concentrated towards the centres of the leaflets. Small necrotic spots may be found in the pale areas. When at the edge of the leaflet these may coalesce into a marginal scorching.

(7) *MacHattie's Giant*

Experimental. Whitsuntide, the only variety of the group tested, showed the most severe necrotic symptoms of the whole series (Pl. XIX, fig. 1).

Field observations. In the MacHattie's Giant group the plants are greatly dwarfed and the leaves very crinkled and mottled.

(8) *Crisp*

Experimental. Iceberg and Webb's Wonderful showed a mild to moderately severe mottle with slight stunting and no necrosis, and were the most tolerant varieties tested (Pl. XIX, fig. 3).

Field observations. In the Crisp varieties clearing of the veins is very evident. The outer surfaces of the leaves assume a hard appearance. Hearting is poor and there is a tendency to shoot prematurely.

HOST RANGE

Experimental inoculations, supplemented by field collections, have extended the host range from lettuce to other members of the Compositae and also to certain leguminous plants.

Attention was paid to certain composite weeds commonly found in lettuce plantings. *Senecio vulgaris* L., groundsel, was found to be susceptible and subsequently diseased specimens were collected from several localities and the presence of the virus proved by inoculation tests. The symptoms shown by groundsel are very mild. About 10 days after inoculation there is a definite, but transitory, clearing of the veins and, later, by careful comparison with healthy plants, a faint mottle can at times be observed. Infected plants are not noticeably stunted, they flower profusely and there is some evidence of seed transmission

(see below, p. 288). *Sonchus asper* Hoffm., prickly sow-thistle, has also been infected and shows a definite leaf mottle but *S. oleraceus* L. could not be infected and no diseased specimens were found in collections of this species and of *S. arvensis* L. *Taraxacum officinale* Web. and *Carduus arvensis* Curt. could not be infected.

It was found that both *Lathyrus odoratus* L., sweet pea, and *Pisum sativum* L., garden pea, were susceptible to lettuce mosaic virus. In sweet pea the virus causes a streak disease while in garden pea a mild mosaic mottling results. In sweet pea there is no apparent reaction for 3–4 weeks, or longer, when severe streak symptoms appear in the stems, usually towards the base, but the initial streaks may be 6 in. or more above soil level, and some shoots may wither and die. If the shoot is not killed the apical leaves remain healthy in appearance or possibly at times show a very mild mottle, but contain a high concentration of the virus. The first symptom in garden pea (var. Lincoln) is a mild vein-clearing about 2 weeks after inoculation and, subsequently, a very mild rather blotchy mottle develops on some leaves of certain plants, but the general effect on this variety of pea has been slight. After experiments had proved that these plants are susceptible naturally infected plants of both sweet pea and garden pea, the former showing streak symptoms, were found in the field. The question of the relation of lettuce mosaic virus to the streak disease of sweet peas in this country is at present under investigation.

Attempts to infect *Lycopersicum esculentum* Mill. (tomato), *Nicotiana tabacum* L. var. White Burley (tobacco), *N. glutinosa* L., *Datura Stramonium* L., *Cucumis sativus* L. (cucumber) and *Brassica oleracea* L. var. *Botrytis cymosa* (cauliflower) failed.

The virulence of the virus to lettuce is in no way reduced by passage through groundsel or garden pea, in which the symptoms are mild.

Transmission

(*a*) *By seed*

Newhall (1923), in Western New York, was the first to demonstrate that lettuce mosaic was seed transmitted. In two trials, he obtained 4·8 and 3·0% average transmission by sowing seed saved from individual mosaic diseased plants of the variety Big Boston and he found 1–3% of mosaic diseased seedlings in field plantings examined at the two- to three-leaf stage. Brandenburg (1928) claimed 8–10% transmission for Punktmosaik while Ogilvie & Mulligan (1934) and Ogilvie *et al.* (1935) recorded 22–37% infected plants in sowings of seed from mosaic-diseased

cos lettuce (as compared with 11% in sowings of commercial seed). Ogilvie's estimates were made on unprotected field plants and therefore included both seed transmission and secondary infection caused by insects.

In order to examine the question of seed transmission, seed, saved from individual mosaic-diseased plants, was sown in seed boxes containing sterilized soil at the rate of fifty seeds per box. Particular care was taken to insure freedom from insects and the seedlings were examined at frequent intervals and any diseased plants at once carefully removed. The sowings were made at intervals during 1937 (see Ainsworth, 1938) and 1938 and the results, including the numbers of control seedlings raised, are summarized in Table II, from which it can be seen that seed transmission occurred in each of the three varieties employed.

Table II. *Seed transmission of lettuce mosaic*

Variety	No. seedlings raised	No. infected seedlings	% infection
Trocadero	952	56	5·6 (*a*) *
	667	55	8·3 (*b*) *
	275	10	3·64
Total	1894	121	6·40
Feltham King	667	41	6·15 (*a*) *
	550	40	7·3 (*b*) *
	64	1	1·57
Total	1281	82	6·4
Lobjoit's Dark Green Cos	276	6	2·17
Grand total	3451	209	6·06
Controls†	862	0	0

(*a*) 1-year-old seed. (*b*) 2-year-old seed.

* Difference between these numbers not significant.

† In addition, no case of mosaic occurred in the hundreds of seedlings raised for experiment from virus-free seed in the same glasshouse.

No symptoms of disease appeared on the cotyledons but diseased seedlings were slightly stunted and could be recognized by a distinct mottling of the second, or third, and subsequent foliage leaves. To prove that the seedlings were attacked by mosaic, healthy lettuce plants were inoculated from diseased seedlings, when they developed typical mosaic symptoms, and tests showed the virus obtained from the seedlings to be similar to that with which the seed plants were infected. It was usual to grow the plants on in the boxes to the seven- to nine-leaf stage, but sometimes representative samples were transferred to pots and grown to a larger size. No plant which appeared healthy at the four- to five-leaf stage was observed to develop mosaic.

Portions of certain samples of the seed saved from infected plants in 1936 were sown in the spring and summer of 1937 as 1-year-old seed, and further portions during 1938 as 2-year-old seed. There was no diminution in the amount of transmission through the 2-year-old seed (see Table II) and mosaic seedlings have been found in sowings of commercial seed known to be at least 3 years old.

An attempt was made to ascertain whether the virus was also transmitted through groundsel (*Senecio vulgaris*) seed. A total of 846 plants was raised from seed saved from experimentally infected groundsel plants, and, because of the difficulty in recognizing mosaic disease in groundsel, 530 of these plants (mostly taken at random) were tested for the presence of virus by the inoculation of juice of individual plants into one to three lettuce seedlings or by inoculating a group of lettuce plants with inoculum prepared by grinding together a number of whole groundsel seedlings or separate leaves from larger plants, with or without sodium sulphite solution. The inoculation tests revealed the presence of three infected groundsel plants and, although so few infected plants were detected, the result is thought to be significant.

Several hundred seedlings were raised from seed taken from infected *Sonchus asper* plants but all appeared healthy.

(*b*) *By insects*

General field observations indicate that spread of lettuce mosaic is associated with infestation by aphides. Jagger (1921) was able to transmit the virus by means of *Myzus persicae* Sulz. and Brandenburg (1928) transmitted Punktmosaik by *Macrosiphum hieracii* v.d.G. At Long Ashton, in 1934, two lots of fourteen lettuce plants were placed under cages in the open and, to each plant of one lot, two *Macrosiphum sonchi* L. were transferred from a mosaic diseased plant on 22 June. On 19 July three of the fourteen plants showed symptoms of mosaic, whilst the fourteen control plants under the other cage were all healthy. Dr K. M. Smith has informed the writers (*in litt.*, July 1938) that he succeeded in transmitting the virus by *Myzus lactucae*, but that this insect did not appear to be a very efficient vector as only about a quarter of the experimental plants developed the disease.

To supplement these observations experiments were performed to test the ability of several different aphides to transmit the virus under controlled conditions. The aphides, kindly identified by Mr F. Laing, Mr E. R. Speyer and Dr C. L. Walton, were either collected from

naturally infested plants, e.g. *Myzus persicae* from carnations, or stocks were raised from a few selected individuals. In most experiments the aphides were starved for an hour or two before feeding on a diseased plant (as advised by Watson, 1936) and the feeding time was relatively short, the insects being caged 15–25 min. on diseased plants, although in some experiments the time on infected plants was extended to 72 hr. The insects were next caged for 24–30 hr. on healthy plants which were then fumigated, set aside, and kept under observation. Five to ten aphides were placed on each test plant. Several independent tests were made with most aphides and the results are summarized in Table III.

Table III. *Transmission of lettuce mosaic by aphides under controlled conditions*

	No. plants inoculated	No. plants infected
Myzus persicae Sulz.	33	15
M. pseudosolani Theob.	35	0
M. hieracii Kalt.	18	0
Macrosiphum gei Koch.	23	1
M. sonchi Linn.	46	0
Rhopalosiphum rufomaculatum Wilson	9	0

Myzus persicae transmitted the virus in each of four independent tests (20–90% transmission), but, of the other aphides used, only *Macrosiphum gei* gave any positive results. The negative results are not sufficiently extensive to disprove the ability of these aphides to act as vectors of lettuce mosaic virus in the field. They only indicate that the aphides in question do not transmit the virus efficiently under conditions favourable for its transmission by *Myzus persicae*. An indication of the efficiency of aphides as vectors in the field is shown by the following experiment. On 30 June ten seedling lettuce plants growing in pots were caged in the field with several mosaic diseased cos lettuces which were infested with aphides. These were mostly *M. hieracii*, but a few individuals of other species may have been present. Ten days later there were very few aphides on the caged plants owing to the activities of parasites, but during the period 18–25 July seven of the ten seedlings developed symptoms of mosaic. The remaining three plants, on which no aphides were found, were transferred to the glasshouse where they remained healthy.

Aphides occurring on lettuces. In the case of the following records of aphides occurring on lettuces the insects were identified by Dr C. L. Walton.

Macrosiphum gei Koch. is by far the commonest aphis on lettuces in

the Bristol Advisory Province. It was found, for example, on lettuces on the following occasions:

Late summer, 1930.	At Long Ashton.
29 March 1932.	At Long Ashton.
15 Oct. 1934.	Fairly numerous throughout the Province.
May 1935.	Very abundant at Charlton, near Evesham, and associated with severe mosaic.
9 Oct. 1935.	Sparsely on seed beds at Evesham. Distinctly mottled plants at this date.
15 Oct. 1935.	On seed beds at Fishponds, Bristol.
19 Nov. 1935.	Very prevalent at Perdiswell, Worcs.
22 May 1936.	Very common at Fladbury, near Evesham.

Macrosiphum sonchi Linn. was found on lettuces as follows:

22 June 1934.	At Long Ashton.
18 May 1938.	Very common on young summer lettuces at Cheltenham.

Myzus lactucae Schr. was found on lettuces as follows:

May 1933.	At Charlton, Worcs.
29 Oct. 1935.	Considerable infestation on old plants of summer lettuces at Cheltenham.
Nov. 1935.	At Perdiswell, Worcs.
4 Mar. 1936.	In glasshouses, Long Ashton.

Myzus persicae, the most efficient transmitter of the disease under controlled conditions, has not been found on lettuces in the Bristol Advisory Province, but was recorded on *Sonchus oleraceus* at Long Ashton on 9 December 1933. Mr C. T. Gimingham, of the Ministry of Agriculture's Plant Pathological Laboratory, informs the writers that *Myzus persicae* is rarely recorded on lettuce in other parts of the country.

Myzus pseudosolani Theob. has been found infesting lettuce under glass in Hertfordshire. The saliva of *M. pseudosolani* is toxic to lettuce seedlings and leaves on which this aphis has fed become very malformed.

Other aphides are occasionally recorded on lettuces.

It must be concluded from the above records that *M. persicae* is not the principal vector of lettuce mosaic in Great Britain and, although the fact that aphides spread the disease in the field is established, the elucidation of the conditions under which transmission occurs requires further investigation.

Field observations

In the course of the Vegetable Disease Survey carried out by Long Ashton Research Station during recent years it has been found that mosaic disease commonly causes considerable loss in lettuce plantings. In Worcestershire the name "rust" has long been applied by growers to a complex of diseases which includes mosaic disease, ring spot (caused by

Marssonina Panattoniana) and *Botrytis* disease. Of these three diseases mosaic is by far the most important.

Presumed causes. The disease has been attributed by growers in the past to faulty manuring, to deficiencies of essential chemicals in the soil, to mixed seed, giving rise to "rogues", and to the direct effects of aphis infestation.

Economic importance. During years when the disease is prevalent it may cause great loss in winter lettuces in the south-west of England. Entire fields may become worthless and have to be ploughed under, and this may be reflected in scarcity of lettuces on the market.

In the spring of 1934 many plantings of winter lettuces failed entirely in the Bristol, Cheltenham and Evesham districts. Counts were made of diseased plants in twenty-four commercial plantings with the following results:

Bristol:	av. 34% infection
Cheltenham:	av. 46% ,,
Evesham:	av. 39% ,,

In the spring of 1935, when mosaic was again prevalent, similar counts gave the following results:

Bristol:	av. 39% infection
Cheltenham:	av. 57% ,,
Evesham:	av. 38% ,,

Seasonal occurrence. Mosaic disease does not usually occur to any marked extent on greenhouse lettuces. Here and there occasional affected plants may be found, but unless aphides are present, which is usually not the case in well-managed greenhouses, there is little spread.

In the west and south-west of England winter lettuces are sown out of doors in August and September to stand the winter. They make little growth during the winter months but grow rapidly during the early spring and are ready for cutting during May.

A few diseased plants may usually be found among the seedlings in the early autumn. These arise, presumably, from infected seeds. It is probable that much spread takes place at this time if the weather is dry and aphides are prevalent. This is indicated by the fact, well known to growers, that much less disease becomes apparent if the seeds are sown later in the open, or in frames in January or February before planting out. Thus in one case at Cheltenham an early autumn planting gave at maturity 66% infection, a late autumn planting 35% infection and a February planting (from frames) 20% infection.

In the west of England the disease is not usually common on the Trocadero and other varieties grown throughout the summer. This is, apparently, because the numbers of aphides soon become reduced by parasites and predators. In seasons when aphides are very prevalent during the summer, however, mosaic disease may be prevalent on summer lettuces also. For instance, in August 1938, 75 % mosaic was recorded on one variety of summer lettuces at Long Ashton.

Relation to weather conditions. Prevalence of mosaic disease in spring on winter hardy varieties of lettuces is usually correlated with dry weather, favourable to the multiplication of aphides, during the previous October and November when the plants were in the seedling stage. This is seen from the data assembled in Table IV, and it is interesting to note that these observations are in line with those of Davies (1935) on the effect of humidity on the activities of aphides on potatoes.

Table IV. *Rainfall at Long Ashton and incidence of mosaic on winter lettuces in the Bristol Advisory Province*

	Rainfall in in. (Oct. and Nov.)	Incidence of mosaic in the following spring
1932	10·50	Slight
1933	3·41	Very prevalent (for % see above, p. 291)
1934	3·53	Very prevalent (for % see above, p. 291)
1935	12·53	Slight (about 5 %)
1936	6·71	Slight (about 5 %)
1937	5·37	Slight

The average total rainfall for October and November during the last 25 years was 7·43 in.

Properties of the virus

The properties of the virus were, with the exceptions noted, determined with preparations of crude lettuce sap prepared by grinding infected lettuce plants in a mortar and then expressing the sap through muslin. Powdered carborundum was added to the preparations before inoculating the test plants.

Resistance to heat. Samples of infected juice were enclosed in thin-walled test-tubes and subjected to various temperatures for 10 min. periods. The results are summarized in Table V, from which it can be seen that the virus is inactivated at a temperature between 55 and 60° C.

Table V. *Resistance of lettuce-mosaic virus to heat*

	Unheated	50° C.	55° C.	60° C.	70° C.
No. of plants infected	27*/39	10/56	1/37	0/47	0/10
% infection	69·3	17·9	2·7	0·0	0·0

* Denominator, number of plants inoculated; numerator, number infected.

Resistance to ageing. The results of the tests on the longevity of the virus are summarized in Table VI.

Table VI. *Resistance of lettuce-mosaic virus to ageing* in vitro

Time in hr. ...	0	6–7	12	24	48	72
No. of plants infected	36*/69	5/48	2/20	7/63	1/48	0/35
% infection	52·2	10·4	10·0	11·1	2·1	0·0

* Denominator, number of plants inoculated; numerator, number infected.

In crude juice the virus survives 48 hr. or less when stored *in vitro* at laboratory temperature. It was detected after 24 hr. in three of seven tests and after 48 hr. in one of five. The rate of inactivation of the virus in expressed sap is rapid. Sap from a mosaic-diseased lettuce was used to inoculate ten lettuce seedlings as soon as it was expressed and then further sets of ten plants were inoculated at intervals of 1, 2, 3 and 6 hr. All the plants of the first set developed mosaic, while in the later series the numbers of infected plants were four, one, two and two respectively. It was thought that, perhaps, the inactivation of the virus might be delayed in the presence of a reducing agent, as is the virus of tomato spotted wilt (Bald & Samuel, 1934; Ainsworth, 1936). It was found that if juice was prepared by grinding the infected material with a little 0·5% sodium sulphite solution the virus could regularly be detected after 24 hr. (up to 80% infection) and more frequently after 48 hr. It was not detected in preparations 3 days old or older. It is, however, probable that the virus could be preserved *in vitro* for longer periods under appropriate conditions.

Filtration. The virus could not be filtered through a thin layer of Kieselguhr or Celite or through an L 1 Pasteur-Chamberland filter, even when filtration was carried out quickly and in the presence of sodium sulphite.

Dilution. The amount of dilution the virus will stand is low. It has been detected at 1/50 with juice diluted with water and at 1/100 if diluted with sodium sulphite solution, while higher dilutions gave negative results. A rough estimate of the virus concentration in plants infected for different periods of time was made by inoculating on one occasion series of ten lettuces with preparations of equal concentration on a fresh weight basis, of plants 14–90 days after inoculation, but no difference in virus concentration was detected.

DISCUSSION

The virus here described has been detected in mosaic diseased lettuces obtained from different localities in this country and has, so far, been the only virus found attacking lettuce in England. The disease is almost

certainly the same as that described by Jagger (1921), and an indication that the American lettuce mosaic is identical with the one found in this country is afforded by the fact that the same virus was found infecting one mosaic diseased seedling which appeared in a sowing of a commercial sample of Big Boston lettuce seed obtained direct from an American seed company. Brandenburg (1928) described two types of mosaic, Blattnervenmosaik and Punktmosaik. The former was seed transmitted to the extent of approximately 20% and could not be transmitted by either mechanical means or aphides, while the latter was transmissible by seed (8–10%) and insects, but no mention is made of its transmission by mechanical methods. It is probable that lettuce mosaic should be identified with Punktmosaik, with which it agrees in general symptoms and behaviour, but the dot mosaic symptom has been less evident than under Brandenburg's conditions. No disease that can be equated with Blattnervenmosaik has been observed but, in certain varieties, lettuce mosaic appears as a vein-banding very similar to that of Blattnervenmosaik (cf. Pl. XIX, fig. 2 with Brandenburg (1928), p. 40, Abb. 2 and 3).

The status of the virus has not yet been fully elucidated, and the question of its relation to the already described leguminous viruses is under investigation. It is probable that the host range could be extended but the hosts recorded in this paper, together with the properties of the virus described, should make future recognition of the virus possible. The virus is quite distinct from that of tomato spotted wilt, which also attacks lettuce (Tompkins & Gardner, 1934) and causes a streak disease in sweet pea (Snyder & Thomas, 1936). Spotted wilt has not yet been recorded on lettuce in Great Britain but experimental inoculations have shown the symptoms to be necrotic local lesions (Pl. XX, fig. 5 A) which, if not the only reaction, are followed by a systemic necrosis of the central leaves (Pl. XX, fig. 5 B), often one sided as described by Tompkins & Gardner (1934), without mosaic mottling. Systemic infection in seedling lettuce has resulted in the death of the plants.

Control measures

Lettuce mosaic can be controlled on the lines applicable to most virus diseases. As healthy as possible a stock of seedlings must be raised and steps taken to ensure that the virus is not spread within the crop or introduced from without. When devising methods for the control of lettuce mosaic it must be remembered that the virus is seed transmitted, aphides are vectors, certain common weeds are susceptible to the disease,

and different varieties of cabbage lettuce vary greatly in their reaction to the virus.

Provided virus-free seed is used, or the seedlings are carefully examined before being transplanted, there appears to be no reason why mosaic should prove a serious disease, difficult to control, in glasshouses and frames where aphides can be eradicated relatively easily. It is in the field, where aphis attack is more difficult to combat, and in situations where weeds are allowed unchecked that mosaic becomes a serious disease. Under such conditions the chief points to which attention must be paid are as follows:

(1) *Clean seed.* Virus-free seed should be sown. No doubt in time seedsmen and growers of lettuces for seed will recognize the importance of roguing out diseased plants and reaping seed from healthy plants only. The feasibility of doing this has been shown in this paper.

(2) *Choice of seed bed and planting ground.* It has been found that less aphis infestation and less mosaic disease is apt to result in hilly and open situations. In low-lying situations surrounded by trees and buildings, infection is likely to be severe, as can be seen from the percentage infection at Cheltenham in comparison with other localities given above (p. 291).

(3) *Time of sowing.* In the case of winter lettuces sown outside to stand the winter, fairly late sowings and sowings in frames tend to reduce aphis infestation and, hence, to give a cleaner crop. Planting out in spring also appears to give less mosaic infection than otherwise.

(4) *Use of insecticides.* The use of nicotine sprays or dusts would no doubt reduce aphis infestation, but the value of these in controlling spread of mosaic infection out of doors is problematical. Nicotine fumigation in glasshouses is effective in reducing aphis infestation.

(5) *Weeds.* Weeds should be eradicated. Special attention must be paid to groundsel for, although not a perennial plant, overlapping generations of this weed occur all the year round, especially in gardens.

(6) *Choice of varieties.* It is recommended that in localities where the disease is troublesome, varieties which show only mild symptoms might be substituted for those which become markedly affected. Where, however, the market value of the lettuce is important, care should be taken to select a variety suitable in colour, size and time of maturing, and one which will grow satisfactorily under the conditions in question.

In Table VII the characters and uses of the different groups of cabbage lettuces are briefly summarized and the reactions of a number of varieties indicated.

Table VII. *Response of lettuce groups to mosaic disease*

Group	Characters	Use	Varieties which show severe symptoms	Varieties which show slight symptoms
(1) Gotte	Leaves and heart rounded	Forcing	Loos Tennisball	—
(2) All the Year Round	Round, leaves rather smooth, green	Open (spring and summer)	All the Year Round, Feltham King	Exceller, Spring Beauty
(3) May Queen	Leaves blistered, hearts loose, lower leaves flat	Forcing, Frames, Open (spring)	May Queen, May King, Winter Victor	—
(4) Trocadero	Hearts good, flat on top. Lower leaves flat. Edges of mature leaves folded	Open (summer)	Unrivalled, Improved Trocadero	Trocadero, Premium, Improved Unrivalled, All Seasons Perfection
(5) Lee's Immense	Leaves dull, smooth edges, yellowish	Open (winter)	Yates's Winter White, Schofield's Winter White, Hardy Winter Green	Hardy Hammersmith, Webb's Immense Hardy Green, Clarke's Imperial, Green Winter, Lee's Immense
(6*a*) Passion	Compact, hardy	Open (winter)	Arctic, Arctic King, Imperial, Majestic, Excelsior	—
(6*b*) Stanstead Park	Large, spreading. Brown spots on leaves	Open (winter)	—	Stanstead Park, Early Spring, Winter Tremont
(7) MacHattie's Giant	Large, spreading, blistered leaves	Open (winter)	MacHattie's Giant, Whitsuntide, Goliath, Best of All	—
(8) Crisp	Leaves curled and blistered	Open (summer)	Webb's Wonderful, Summer Standwell	—

Summary

Previous records of lettuce mosaic are cited. The symptoms of the disease on lettuce are described, and the variation in the reaction of different varieties of cabbage lettuce indicated. The host range is extended to certain composite weeds (including groundsel, *Senecio vulgaris*) and to members of the family Leguminosae (*Pisum sativum*, *Lathyrus odoratus*). Experiments in which the virus was seed transmitted through lettuce to the extent of about 6% are described, and the results of experiments with aphis vectors reported. An account of field observations on the influence of the weather on the disease is given. Certain properties of the virus are described and the status of the virus is discussed. Control measures are suggested.

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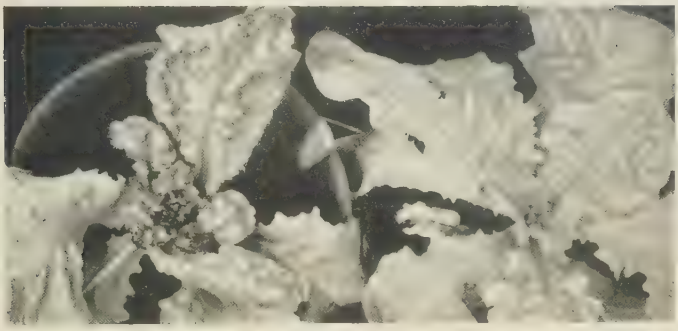

Fig. 1.

Fig. 2.

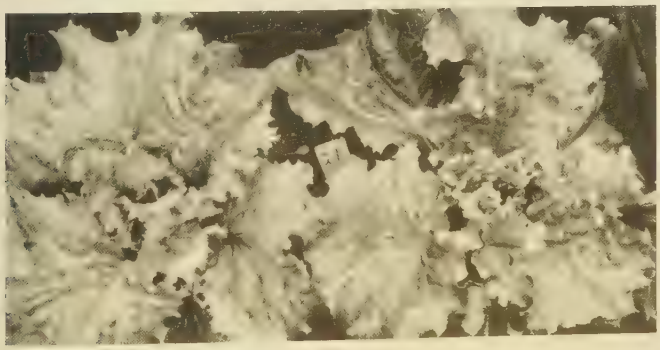

Fig. 3.

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EXPLANATION OF PLATES XIX AND XX

PLATE XIX

Fig. 1. Cabbage lettuce, var. Whitsuntide, showing symptoms of mosaic 17 days after inoculation. (Healthy control plant on right.)

Fig. 2. Cabbage lettuce, var. May King, showing symptoms of mosaic 16 days after inoculation. (Healthy control plant on right.)

Fig. 3. Cabbage lettuce, var. Iceberg, showing symptoms of mosaic 36 days after inoculation. (Healthy control plant on right.)

(The photograph for Fig. 1 was taken with a green filter to emphasize the necrosis and that for Fig. 2 with a red filter to emphasize the yellow vein-clearing, hence the apparent large variations in leaf colour.)

PLATE XX

Fig. 1. Cabbage lettuce, var. Cheshunt Early Giant, showing symptoms of mosaic 36 days after inoculation. (Healthy control plant on left.)

Fig. 2. Necrotic lesions on stem and bracts of a "bolted" Cheshunt Early Giant plant.

Fig. 3. Cos lettuce, var. Lobjoit's Dark Green Cos, showing symptoms of mosaic. (Healthy control plant on left.)

Fig. 4. Cos lettuce, var. Demi Plate Maraîchère, showing mosaic symptoms.

Fig. 5. Spotted wilt on lettuce, var. Cheshunt Early Giant: A, local lesions 10 days after inoculation; B, systemic necrosis after 18 days.

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EXPERIMENTS AND OBSERVATIONS ON A VIRUS DISEASE OF WINTER SPINACH (*SPINACIA OLERACEA*)

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(With Plate XXI)

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Introduction

During the winter of 1936 the writer's attention was drawn to a disease of winter spinach occurring at Slough. Reports from growers indicated that the disease was prevalent in the neighbourhood, and that the intensity varied from season to season.

Winter spinach is sown from the third week in August till about the first week in October, and is usually left until the following spring, when picking commences and continues till the middle of May. The earliest sowings may be given one or more pickings before the winter sets in and then left until the spring.

Plots were laid down in the autumn of 1936 to ascertain if the condition was due to a mineral deficiency. Two plots were treated with sulphate of potash at the rate of approximately 4 cwt./acre, two with borax at the rate of 20 lb./acre, and two were left untreated as controls. The amount of disease on the various plots bore no relation to the treatment, and on investigating the problem along different lines it was found

that a virus disease was concerned. It is not to be assumed that the diseases of spinach noticed from time to time by growers are all of this nature: waterlogging of the soil and mineral deficiencies may play a part.

Historical

The *List of Common Names of British Plant Diseases* (1929) contains no reference to a virus disease of spinach. Dillon Weston (1934) records the occurrence of spinach mosaic in Bedfordshire. The virus nature of the disease was confirmed by Dr K. Smith (quoted by Dillon Weston, 1935), but no indication is given as to the relationship of the virus principle concerned.

A virus disease of spinach, called "spinach blight", was first recorded from America by McClintock & Smith (1918). It is characterized by a mottling and malformation of the leaves, by a decided stunting of growth and by premature death of the plant. The disease is transmissible by needle inoculations and by aphides. Boning (1927) has recorded the presence, in the neighbourhood of Bonn, of a disease very similar to the above and shown that the virus is transmissible to mangolds by insects but not by mechanical inoculation. Volk (1929) describes the occurrence of an epidemic of spinach mosaic in the foothills between Bonn and Cologne, losses in many places amounting to 90–100%. The general impression amongst German workers is that the disease recorded from Germany is identical with the American disease, but the description given by Boning is slightly different from that recorded by McClintock & Smith. No attempt was made to identify the virus responsible for the disease in Germany.

Hoggan (1929) showed that the virus causing cucumber mosaic is transmissible to spinach and that the symptoms produced are very similar to those described for spinach blight. Later (1933) she demonstrated that spinach is susceptible to at least three different viruses, namely those of cucumber and sugar-beet mosaic, and the tobacco ring-spot virus. The first two are frequently responsible for the death of the plant. The symptoms produced by sugar-beet mosaic on spinach differ from those produced by cucumber mosaic in that there is no fine discrete spotting of the leaves as a primary symptom, and no malformation of the leaves occurs later. As a result of her investigations Hoggan considers that the American spinach blight is due to cucumber mosaic virus. This result is also supported by the isolation of the virus from naturally diseased material, whilst she tentatively suggests on the basis of the published descriptions that the German disease is due to the virus of

sugar-beet mosaic. Wilhelm (1935), however, showed that the virus responsible for the mosaic of spinach in the neighbourhood of Bonn is transmissible to cucumber and vice versa. Furthermore there is no record of sugar-beet mosaic infecting cucumbers.

Smith (1937) records infection of spinach by sugar-beet mosaic, sugar-beet curly-top virus and *Callistephus* virus. Tobacco-mosaic virus has been described by Jones (1934) as infecting spinach and killing the plant.

Description of the disease

During the winter of 1936–7 observations on the disease were made at Slough, on the plots mentioned. Individual plants showing early symptoms were selected and observations made on the course of the disease until the death of the plant. At any time throughout the winter, plants showing all stages of the disease could be found and Pl. XXI, figs. 1 and 2 show typical examples of diseased and healthy plants obtained from the field and potted.

The first visible symptom of the disease in the field is a yellowing of the younger leaves, which becomes more pronounced, and spreads to the outer leaves as the plants become older. At this stage the plant becomes stunted and the young leaves are distorted, their edges being cut and feather-like. The outer leaves become limp, and the tips lie on the ground. Eventually, the whole leaf lies on the ground, by which time the outer leaves have lost most of their green colour. The last part of the leaf to remain green is usually that bordering the veins (Pl. XXI, fig. 2 B).

Disintegration of the older tissues begins, starting at the tips of the leaves, and working its way inward until only the petioles remain (Pl. XXI, fig. 1 D, E). The plant is reduced to a small clump of much distorted leaves 1–2 in. in diameter, and later the rotting spreads to the inner leaves and the plant dies. During the winter of 1936–7 the rotting of the tissues was much more rapid than in 1937–8, when owing to an exceptionally dry spell the progress of disintegration was delayed and the leaves became brown and shrivelled. The root of the plant remains in healthy condition for a considerable time, and begins to disintegrate only during the final stages of the disease.

The time taken for these changes to occur varied from about 50–64 days after the appearance of the first symptoms. In experimentally inoculated plants the time from inoculation to death varied from 80 to 96 days, the first symptoms appearing about 20–30 days after inoculation.

An attempt was made to obtain seed from diseased plants, but the majority of them had died before the time of flowering. Some plants which became infected later in the season sent up short distorted shoots, and in one or two plants seed was set. However in most cases the embryo was small and shrivelled.

Inoculation studies

The cause of the disease and the relationship of the active principle were determined as follows:

Spinach. (*a*) Twelve spinach plants, chosen at random in a field crop which had been sown in the previous August, were inoculated in February by rubbing juice from diseased plants on to the leaves with the pestle which had been used for grinding up the tissues. Twenty-four plants, one on either side of those inoculated in the row, were similarly rubbed with water to serve as controls. Eleven of the former developed typical symptoms of the disease, whereas all the controls appeared healthy after three months.

(*b*) Twelve plants which had been grown in the greenhouse were inoculated as in the previous experiment, twelve others serving as controls. Apart from a tendency to develop the flower axis, due to the forcing conditions in the greenhouse, the inoculated plants all developed the typical symptoms. Control plants remained healthy.

(*c*) Seedling spinach plants in the greenhouse were inoculated at the time when the first two rough leaves had developed. Of thirty-two such plants, fifteen developed the disease. The infected plants showed yellowing and malformation of the younger leaves and finally died. Pl. XXI, fig. 3 illustrates a typical example of a diseased and a control plant 8 weeks after inoculation.

Cucumber. Inoculation of young cucumber plants by rubbing their surfaces with sap from diseased spinach gave positive results with about one-half of the plants tested. Infected plants became stunted and the young leaves developed a yellow mottle. This mottling is characterized by the formation of dark green raised areas and the whole leaf, therefore, gives the impression of abnormal greenness. There is some distortion of the leaves and the internodes of the stem are reduced. Fruit is set less readily than in the control plants and diseased fruits show a yellow mottle.

Tobacco. The symptoms were very mild and showed only when the plants were rapidly growing. The first symptom was a slight clearing of the veins, followed by a mild mottle.

Nicotiana glutinosa. The symptoms were more definite than those produced on tobacco. The leaves became mottled and somewhat distorted. In one plant necrotic lesions were produced at the tip of some of the younger leaves. The plants became stunted.

Tomato. No mottling or distortion of the leaves could be detected, and the only symptom was an abnormal tendency for the lateral buds to develop.

No successful inoculations were obtained with expressed sap which had been stored in the laboratory for 2 days.

Of the six viruses which are known to produce symptoms in spinach four may be eliminated in the case of the disease under consideration.

Sugar-beet curly-top virus is not easily sap-inoculable, and *Callistephus* virus I can be transmitted by insect vectors only. The symptoms produced by the spinach virus on tobacco and *Nicotiana glutinosa* differ from those described by Smith (1937) as typical of tobacco mosaic and tobacco ring-spot virus. The former produces local lesions on *N. glutinosa* whilst the latter gives ring-like patterns on tobacco. The causal agent must, therefore, be the virus of sugar-beet mosaic or of cucumber mosaic or an unrecorded virus. Table I gives a comparison between sugar-beet mosaic, cucumber mosaic I, and the spinach virus.

Table I. *Comparison of sugar-beet mosaic, cucumber mosaic I and spinach virus*

	Sugar-beet mosaic	Cucumber mosaic I	Spinach virus
Longevity *in vitro*	24–48 hr.	72–96 hr., 48–72 hr. (Ainsworth, 1935)	24–48 hr.
Transmission	Transmitted by hard rubbing	Easily transmitted by sap	Easily transmitted by sap
Spinach	Yellow flecks on young leaves, no marked malformation; necrosis of outer leaves followed by death of plant	Uniform chlorosis and malformation of young leaves; necrosis of older leaves followed by death of plant	Uniform chlorosis and malformation of young leaves; necrosis of outer leaves followed by death of plant
Tobacco (mechanical inoculation)	Not infected	Slight clearing of veins followed by mild mottle	Slight clearing of veins followed by mild mottle
Nicotiana glutinosa	Not infected	Mild mottle and distortion of leaves; stunting of plant	Mild mottle and distortion of leaves; stunting of plant
Cucumber	Not infected	Yellow mottle and distortion of leaves; stunting of plant; fruit mottled	Yellow mottle and distortion of leaves; stunting of plant; fruit mottled
Tomato	Not infected	Mild mottle without distortion and slight narrowing of the leaves; "Fern leaf" produced under certain conditions; Mogendorff (1930) records production of lateral leaflets and shoots	No leaf distortion or mottle; tendency for lateral shoots to develop

There is thus a great similarity between the virus of cucumber mosaic and that of the spinach disease. The only differences noted were that the spinach virus had a shorter period of longevity, and that rather different symptoms were produced on tomato. However, the period of longevity is of the same order, and it was shown by Ainsworth (1935) that

the longevity of cucumber mosaic was rather variable. In only two out of eight tests recorded by Ainsworth was the virus active on the third day.

The symptoms produced on tomato by cucumber mosaic have also been shown to vary considerably. "Fern-leaf" symptoms, according to Mogendorff (1930), are produced only occasionally when the virus is inoculated artificially into tomato plants. The prolific growth of the axillary buds and the branching of the main stem are described by him as being caused by the virus, but neither Ainsworth (1935) nor Smith (1937) records these symptoms.

Inoculations were carried out with the virus obtained from spinach and with authentic cucumber mosaic I obtained from Dr Ainsworth. The viruses were inoculated on to spinach, tobacco, *Nicotiana glutinosa* and cucumber. Identical symptoms were produced on the last three hosts, but spinach tended to flower so rapidly, with consequent withering of the leaves, that no definite symptoms developed.

The results of the above inoculation studies indicate that the disease is caused by cucumber mosaic I, and that it is probably identical with the disease recorded by McClintock & Smith (1918) from America.

Field studies

Observations were made during the season of 1937–8 on the occurrence of the disease in the field. Various growers in the Thames Valley were visited and the disease was found to be fairly severe at three centres. Estimates of the intensity of the disease were made by taking sample counts, and the loss due to the disease was shown to be 19, 13 and 40–50% of the total stands at the three farms. The following factors appear to be important in the incidence of the disease.

(*a*) *Time of sowing*

In the autumn of 1937 an experiment was carried out at Slough to ascertain if the date of sowing affected the severity of the disease. Sowings were made on 4 August, 18 August and 1 September, four plots (10×10 ft.) being sown on each occasion. The plots were situated in one field, and the replicates were randomized as far as the position of the plots would allow. Table II gives the number of diseased plants present on the plots, at intervals of two months, throughout the season. Germination was good in all the sowings, but the final stand on some of the earliest sown plots was poor owing to the date of sowing being too early, so that many plants flowered before the winter set in. Frost also killed some of the earliest sown plants.

Table II. *Effect of date of sowing on the occurrence of the disease at Slough*, 1937–8

Plot no.	Date of sowing	No. of diseased plants on 6 Oct.	13 Dec.	10 Feb.	10 April
1	4 Aug.	1	7	12	19
2	,,	5	8	20	7
3	,,	2	12	15	27
4	,,	37	54	*	*
5	18 Aug.	1	0	2	0
6	,,	0	0	2	5
7	,,	0	2	8	12
8	,,	0	0	2	8
9	1 Sept.	0	0	0	0
10	,,	0	0	0	0
11	,,	0	0	0	0
12	,,	0	0	8	2

* Plants affected by frost.

Table II shows the progress of the disease as the winter advanced. No account was taken of plants which had died and disappeared, and this explains the low numbers of diseased plants present in some plots on the last date of recording. Table II also shows that the earlier the sowing the greater the incidence of disease.

(*b*) *Aphis infestation*

Samples of seventy plants taken from each plot were examined for the presence of aphides. The results are given in Table III, which shows the numbers of plants infested with winged and wingless forms.

Table III. *Aphis infestation on* 15 *September* 1937

Plot no.	Date of sowing	No. of plants infested with aphides: Winged	Wingless	% infestation
1	4 Aug.	2	8	14
2	,,	8	6	20
3	,,	1	9	14
4	,,	4	35	54
5	18 Aug.	1	0	1·5
6	,,	1	6	10
7	,,	1	3	6
8	,,	1	5	8·5
9, 10, 11, 12	1 Sept.	0	0	0

Table III indicates a higher degree of aphis infestation in the earlier sowings, and a comparison with Table II shows that aphis infestation is correlated with the amount of disease present on the plots. Plot 4, which had the highest percentage infestation, developed

most disease, whilst the last sowings showed no aphis infestation and negligible disease. The aphis population declined rapidly after the middle of September, and very few could be found on the spinach at Slough from November onwards, and into the late spring. This would account for the relatively small amount of spread which was shown in the Slough plots during the winter. On the other hand, at three of the farms visited, the disease continued to spread throughout the winter, chiefly along the rows where the plants were in contact. At two centres there was also a considerable amount of lateral spread, so that diseased patches up to 3–4 ft. in diameter were seen. These showed at the edges plants in various stages of the disease, whereas in the centre all traces of the plants had gone. At these farms there was in the spring a considerable infestation of the crop with aphides, and it may be presumed that a certain population of aphides had overwintered on the crop.

(c) *Sources of infection*

In order that a crop may become infected by means of aphides, there must be a source of virus inoculum in its vicinity. This may consist of seedlings derived from virus-infected seed or of one or other of the alternative host plants of the virus.

Seed-borne transmission. Seed was collected from diseased and healthy plants during the summer of 1937. Great difficulty was met with in obtaining seed from infected plants, and it was only from such as had become infected in the spring that any seed was obtained. Table IV indicates the weight and percentage germination of seed from diseased and healthy plants.

Table IV. *Comparison of seed from diseased and healthy plants*

Source of seed	Weight of 100 seeds (g.)	% germination
Artificially inoculated	0·21	10
Control	0·42	85
Naturally diseased	0·26	15
Control	0·57	90

The low percentage germination of seed obtained from diseased plants materially reduces the risk of seed-borne transmission. The few plants which were raised in the greenhouse from this source showed no trace of disease after 5 weeks' growth, so that the evidence for the moment is that seed transmission does not occur. However, this possibility should not be ruled out, as the experiment was not on a sufficiently extensive scale, and it may be that diseased seed is produced only when the parent plants become infected at a definite stage in their development.

(*d*) *Other host plants*

Cucumber mosaic virus is known to be able to infect a large number of plants. Smith (1937) gives a list of 100 such species extending over thirty-two families. Attempts were made during the summer of 1937 to obtain naturally diseased material.

Vegetable marrows were observed on a farm showing typical symptoms of a virus disease. Sap from these plants was inoculated into cucumber plants and typical symptoms of cucumber mosaic developed. Summer spinach was also found to be infected. These alternate hosts are probably the chief sources from which infection spreads to spinach in the autumn.

Cucumbers have been recorded from Germany as probably being the chief alternate host of the virus, and at two centres in the Thames Valley, where the disease was worst, a crop of vegetable marrows had been grown within a quarter of a mile of the winter spinach. Both crops were present in the field at the same time, and thus it would be possible for the disease to pass directly from marrows to spinach in the autumn, provided suitable insect vectors were present.

Weed hosts were also examined for the presence of virus, especially *Bryonia dioica*, but naturally infected material of the latter could not be found.

Control measures

In view of the wide host range of cucumber mosaic I, control of the spinach disease will depend partly on the removal of plants carrying the virus in the vicinity of this crop. Vegetable marrows have been shown to be capable of being infected, and spinach should not be planted in their neighbourhood. However, in order to establish definitely that the disease is contracted by spinach from marrow further investigations are necessary on aphis migration from marrow to spinach.

Experiments and observations show that the disease is worst in early sown crops, and an obvious method of control is to postpone the date of sowing till the latter half of September. Since the disease is readily transmissible by mechanical means, there is a serious risk of its being carried along the rows on the hands of workers. This would apply especially when—as is sometimes practised—an early sown crop is picked in the autumn and then allowed to stand over the winter for spring pickings. To compensate for the loss of the autumn picking a crop could be sown in the late summer and, after picking in the autumn, be ploughed in.

Summary

A disease of winter spinach common in the Thames Valley has been shown to be of a virus nature. It is characterized by a mottling and malformation of the leaves, stunting of growth and death of the plants. The causal agent has been shown to be identical with cucumber mosaic I of Ainsworth.

The disease is most prevalent in the earlier sown crops, and this appears to be correlated with their liability to be infested with aphides. Field observations on a number of farms indicated a correlation between intensity of aphis infestation and intensity of disease.

Seed-borne infection, though possible, has not been proved.

Under field conditions in the Thames Valley it is suggested that a main source of contamination of winter spinach is the outdoor vegetable marrow crop.

Postponement of date of sowing until the latter half of September is suggested as a method of reducing the amount of disease.

The writer is indebted to Prof. W. Brown for handing over to him the records of preliminary experiments and observations relating to this disease; also for general supervision and advice in the course of the work.

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EXPLANATION OF PLATE XXI

Fig. 1. Spinach, from field: A, healthy; B–E, progressive stages of disease.
Fig. 2. Spinach: A, healthy; B, naturally infected.
Fig. 3. A, spinach artificially infected in the greenhouse by rubbing leaves with sap from diseased plant; B, control.
Fig. 4. Cucumber leaves: A, control; B, inoculated with virus from spinach.

(*Received* 16 *November* 1938)

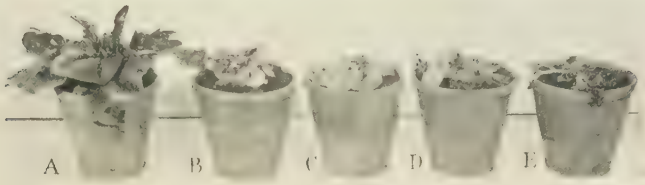

Fig. 1.

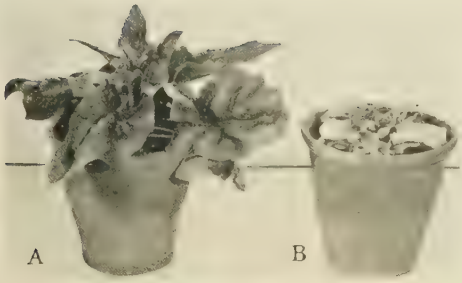

Fig. 2.

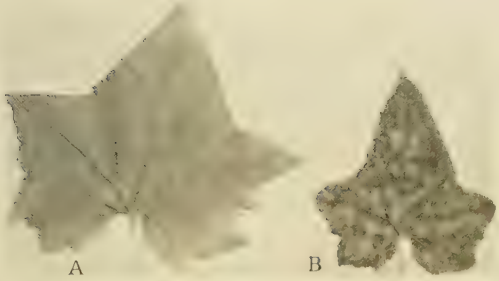

Fig. 4.

Fig. 3.

STOREY.—Experiments and Observations on a Virus Disease of Winter Spinach (*Spinacia oleracea*) (pp. 298–308)

A GAMASID MITE (*TYPHLODROMUS THRIPSI* N.SP.), A PREDATOR OF *THRIPS TABACI* LIND.

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(With 15 Text-figures)

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Introduction

For about 10 years *Thrips tabaci* has been bred on cotton plants grown in a glasshouse at the Manchester University Experimental Grounds so that observations on the biology of this insect could be carried out. It was noticed that, under glasshouse conditions, the natural enemies of the thrips were very few. No parasites were observed and the only important predator appeared to be a gamasid mite. The first appearance of thrips on the plants occurred very regularly about the first week in May each year, and the first mites were generally observed by the end of June. After this time the mites could be obtained in fairly large numbers until the end of November, by which time the thrips had almost disappeared for the season.

The gamasid, which has been identified for me by Dr H. Graf Vitzthum as a new species of *Typhlodromus*, bred very readily under laboratory conditions. Owing to the small size of the mites, especially in the early stages, they were kept in small glass tubes, $1{\cdot}5 \times 0{\cdot}25$ in. It was most important that the corks by which the tubes were closed had no holes or cracks as the mites were able to squeeze into very small cracks and were thus liable to be transferred from one tube to another by mistake. The gamasids were bred at ordinary laboratory temperature and also in an incubator with a constant temperature of approximately 27° C. The latter, however, though giving optimum conditions for development of *Thrips tabaci*, appeared to be too hot to give good results with the mite.

Notes on the biology of *Typhlodromus thripsi* n.sp.

The adult mite is an active creature. There are no eyes, and the first pair of legs appears to be used entirely as tactile organs and not for locomotion. The mite runs rapidly over the leaves of the plant, and when it encounters a thrips larva seizes it at the junction of the thorax and abdomen. The thrips usually lashes its abdomen from side to side in an attempt to shake off the mite, but seldom succeeds if the latter has really gripped the insect. One mite may kill five or six thrips in a day. The young stages, with the exception of the first stage, the larva, are also very active, but the newly hatched mite tends to remain in the place where the egg was laid until after the first moult. It is doubtful if the larval mite feeds.

At laboratory temperatures copulation takes place, on an average, 14 days after the egg has hatched. The shortest time between hatching and copulation was 8 days and the longest 29 days, though it is possible that in the latter instance the mites had already copulated without being observed. Copulation is a prolonged process and may last for some hours. During the process the female runs about the plant in her usual manner with the male attached to her under-side, so that the ventral surfaces are in contact with each other. One female will copulate frequently during her adult life.

At a constant high temperature of 27° C. the interval between hatching and copulation has been as short as 3 days.

Oviposition begins 2–26 days after copulation, but the average time is 12 days. Only one or sometimes two eggs are laid at a time. The female mite lays them at random on the surface of the leaf, with very little attempt to find a sheltered spot, except that as a rule the eggs are found on the under-side of the leaf rather than the upper. After one egg has been laid she usually lays the later ones beside it so that eventually groups of eggs are formed.

The first moult takes place a few hours after the larva has hatched from the egg at 27° C., but at ordinary temperatures it may be delayed as long as 3 days.

Description

Type specimens have been sent to the British Museum.

Typhlodromus thripsi n.sp. *Adult female* (Fig. 1).

Idiosoma:	Length approx.	... ...	0·4 mm.
	Width approx.	... ...	0·24 mm.

Legs:	1st pair. Length approx.	...	0·34 mm.
	2nd pair. Length approx.	...	0·26 mm.
	3rd pair. Length approx.	...	0·27 mm.
	4th pair. Length approx.	...	0·36 mm.

The mite is oval in shape, a little broader at the hind-end and flattened dorso-ventrally. The colour is a light yellowish brown. Under a low magnification the dorsal surface looks quite smooth and polished, but under high magnification a few scattered setae can be seen. The dorsum is almost covered by a plate of chitin, the dorsal shield (Fig. 2). This appears to be single, though a slight constriction about the middle suggests that it may have been formed by the fusion of two plates. There are eighteen pairs of setae, one pair very short, situated in the middle of the anterior border and directed forward over the gnathosoma, one pair, also short, right at the posterior end, and the other sixteen pairs, which are longer and directed backwards, arranged in two series, ten pairs on the anterior and six pairs on the posterior half of the dorsal shield.

On each side below the dorsal plate is a stigma with the peritreme (Fig. 3). The stigma is just posterior to the coxa of the third pair of legs, and the peritreme, which is narrow and slightly undulating, runs forward from it almost to the base of the gnathosoma.

Ventral side. The tritosternum (Fig. 4) is long; the basal piece extends from the anterior border of the sternal plate to the base of the gnathosoma, and the two free ends are approximately the same length as the basal piece.

The sternal plate is nearly square, but the anterior border is convex, with the anterior angles drawn out into points between the coxae of the 1st and 2nd pairs of legs. This plate bears three pairs of setae—one pair at the anterior end, one pair opposite the coxae of the 2nd leg, and the third pair in the posterior angles. The last pair, and the parts of the plate on which they are situated, are separated from the rest of the plate by a faint suture. At the base of the coxae of the 3rd pair of legs are two very small metasternal plates each bearing one seta. The genital plate, which lies in the middle line behind the metasternal plates, bears one pair of setae on the posterior half of the sclerite; the genital opening is on the extreme anterior border and is marked by a thickening of the chitin at this point, and by a peg-like structure projecting forward from it. Under high magnification this peg is seen to be the end of an apodeme, about half of which is inside the body under the genital plate, while the other

half projects forward forming the peg. The genital opening round the peg is fringed with long hairs (Fig. 5). The anal plate is more or less pear-shaped, with the broader end anterior; the anal opening is triangular and occurs near the posterior end of the plate. The anal plate bears four pairs of setae and a single post-anal one. The whole sclerite is traversed by a series of fine striations.

Legs. The 1st and 4th pairs are the longest and are approximately of the same length (0·34 and 0·36 mm. approx.). The 2nd and 3rd pairs are also almost equal in length to each other (0·26 and 0·27 mm. approx.), but the 2nd pair are slightly thicker than the others. The first pair of legs have a larger number of setae than the other three pairs, especially on the distal segments. This is probably due to the fact that the first pair are used exclusively as tactile organs and not for locomotion.

In a preparation of the adult female the "annulated tubes" are conspicuous. These are part of the genital system and appear as a pair of short, thick tubes. One end of the tube is narrow and rounded and appears to have no aperture. There is a tendon attached to the apex of the tube, which is also attached to the synarthodial membrane of the 3rd coxa. At the other end the tube widens out, and in many preparations a round sac is attached to this end of the tube, but this sac is not always visible. In this species the tubes show no signs of annulations of any kind, although by most authors they are described as "annulated tubes" (Fig. 6) (Banks, 1905; Michael, 1892; Vitzthum, 1931).

Gnathosoma (Fig. 7). The mandibles are fairly long and stout, and without hairs. The fixed and movable digits (tibia and tarsus) are equal in length; the fixed digit has a few small teeth.

The coxae of the maxillae form the greater part of the ventral side of the gnathosoma and are divided from each other by a groove, the hypopharynx, running along the midventral line, this groove being partially covered by the arms of the tritosternum. The maxillary palps have the typical five segments and are furnished with hairs which are specially numerous on the two distal segments. The corniculi of the maxillae are well marked, and at their base are three pairs of setae, the setae maxillaris interiores anteriores, the setae maxillaris interiores posteriores and the setae maxillaris exteriores. The maxillary coxae also bear a pair of bristles. There is a very slender sclerite running forward from the hypopharynx between the corniculi of the maxillae.

Adult male (Fig. 9). Only the characters by which the male *Typhlodromus* differs from the female are mentioned.

Idiosoma: Length approx.	...	...	0·30 mm.
Width approx.	...	...	0·17 mm.
Length of legs: 1st pair	...	...	0·28 mm.
2nd pair	...	...	0·23 mm.
3rd pair	...	...	0·23 mm.
4th pair	...	...	0·30 mm.

Ventral side. There is a single sternal-metasternal-genital plate on the ventral side; it is long and narrow and bears five pairs of setae, the same number as is found on the separate plates in the female, placed at the bases of the four pairs of legs and in the posterior angles of the sclerite. The genital opening is at the anterior end, just posterior to the tritosternum, and is shaped like the neck of a flask. The anal plate is broader than that of the female, and while it also has four pairs of setae and a post-anal one, their arrangement is different (Trägårdh, 1911).

Legs. The 4th pair of legs is equal in length to the idiosoma and the 1st pair is nearly as long; the two middle pairs are shorter.

Gnathosoma (Fig. 8). The mouth-parts are similar to those of the female, except that the mandibles have a "spermatophore carrier". This is a spine arising from the outer side of the movable digit of the mandible. The spine projects forward in front of the mandible and its free end is forked. It serves to convey the spermatophores from the male genital opening and insert them into that of the female (Michael, 1892; Oudemans, 1915).

The egg. The egg is an oval, translucent body, approximately 0·2 mm. in length by 0·12 mm. at its broader end. The variation in its width is slight. It is colourless and without sculpturing.

Larva (Fig. 10). The egg hatches into a six-legged larva. This is completely colourless and is not so flattened dorso-ventrally as the later stages. Except that there are only three pairs of legs, the general form does not differ greatly from the adult, but the arrangement of the plates on the dorsal and ventral sides is different. The dorsal plate is divided by a suture into two unequal parts; the anterior part is larger and bears nine pairs of setae; there is one pair at the anterior edge of the plate, directed forwards as in the adult, but most of the setae are longer and stouter in proportion than in the adult, and a particularly long, strong pair projects laterally between the 2nd and 3rd pairs of legs. The posterior part of the dorsal plate has only two pairs of bristles, a short pair near the anterior margin, and a very long, curved pair on the extreme posterior border (Fig. 11).

On the ventral side there are two plates (the anterior one resembles the sternal-metasternal genital plate of the adult male, but has only three pairs of setae) and the anal shield, also similar in shape to that of the adult male, but bearing six pairs of setae as well as the post-anal one.

The mandibles (Fig. 12) resemble those of the female mite; a spermatophore carrier is never present.

No trace of the stigma or the peritreme could be found.

Protonymph (Fig. 13). After the first moult the mite has four pairs of legs and is known as a protonymph.

The dorsal plate (Fig. 14) still shows traces of a division; the anterior half bears eleven pairs of setae, and the posterior eight pairs. The pair of setae right at the posterior end of the plate are very much shorter than in the larva.

Only two plates can be distinguished on the ventral side, and these are similar to the ventral plates in the preceding stage, except that on the anal plate the setae are reduced in number to four pairs and a single post-anal seta, as in the adult mite. The stigma and the peritreme can be seen in this stage, the stigma situated, as in the adult, just anterior to the fourth coxa, but the peritreme (Fig. 15) is very short, only reaching to the base of the third pair of legs.

The mandibles are like those of the larva.

Deutonymph. In this stage the anterior plate on the ventral side is almost the same as in the adult male, and bears five pairs of setae, but there is no genital aperture. The peritreme is longer than in the protonymph but not so long as in the adult. The mandibles are similar to those of the adult female.

Summary

1. *Typhlodromus thripsi* n.sp., of which a description is given, is a predacious mite feeding on *Thrips tabaci* Lind. It appears to be one of the few natural enemies of this insect.

2. There are five stages in the life cycle of the mite; these are: egg, larva, protonymph, deutonymph and adult.

3. The mites first appear in June and are found on the plants until December, by which time the thrips have disappeared for the season.

I would like to take this opportunity of thanking Dr H. Graf Vitzthum for his kindness in identifying the mite, and to thank Prof. H. Graham Cannon, F.R.S. and Dr H. W. Miles for their very helpful criticism and advice during the preparation of this paper.

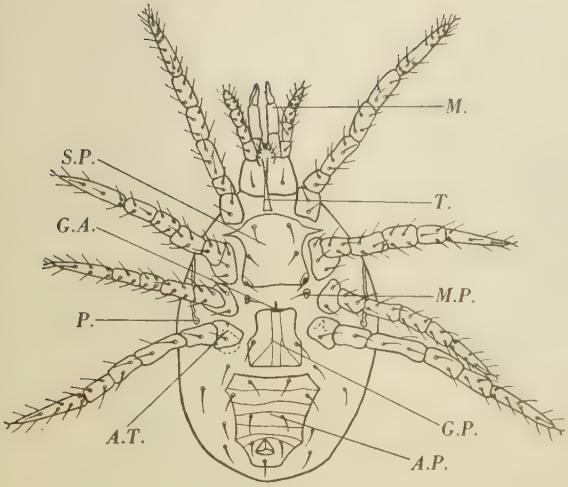
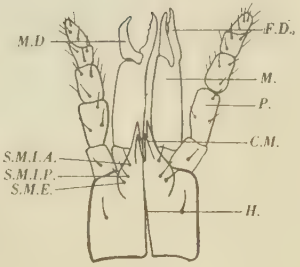


Fig. 1

Fig. 3



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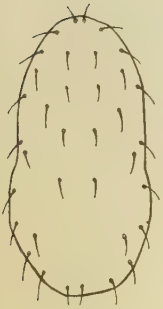

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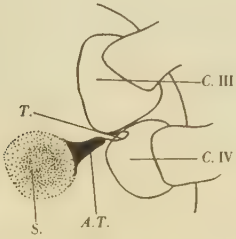

Fig. 6

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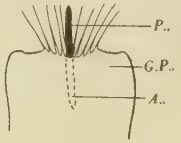

Fig. 5

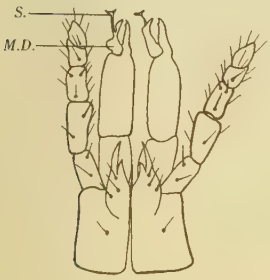

Fig. 8

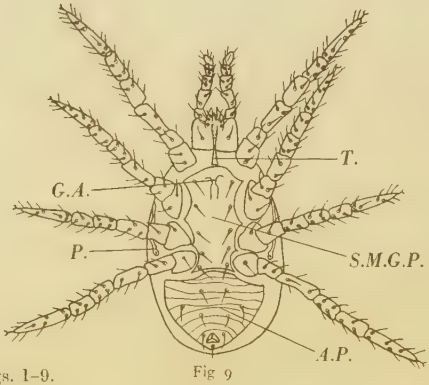

Fig 9

Figs. 1–9.

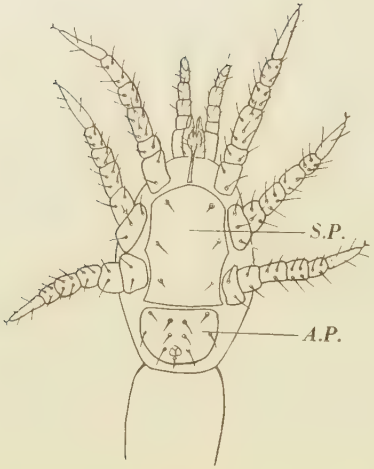

Fig. 10

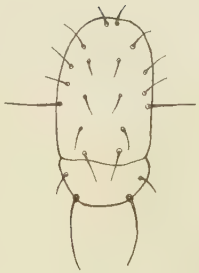

Fig. 11

Fig. 12

Fig. 15

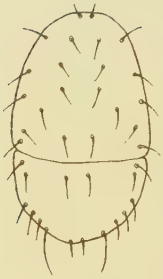

Fig. 14

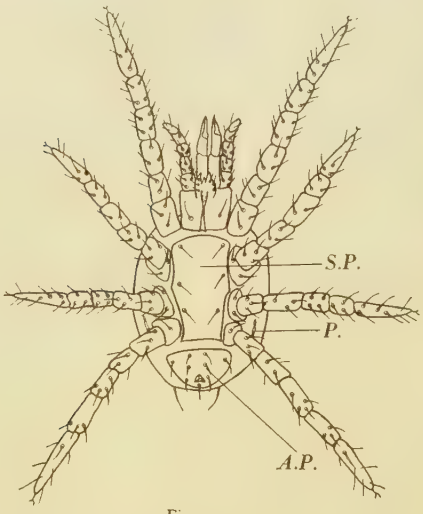

Fig. 13

Figs. 10–15.

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EXPLANATION OF FIGS. 1–9.

Fig. 1. Adult female. Ventral side. × 100. *A.P.* anal plate; *A.T.* position of "annulated tube"; *G.A.* genital aperture; *G.P.* genital plate; *M.* mandible; *M.P.* metasternal plate; *P.* peritreme; *S.P.* sternal plate; *T.* tritosternum.

Fig. 2. Dorsal shield. Adult. × 110.

Fig. 3. Peritreme. Adult. *S.* stigma. × 240.

Fig. 4. Tritosternum. × 330.

Fig. 5. Genital aperture. Female. × 740. *A.* part of apodeme under genital plate; *G.P.* genital plate; *P.* "peg"—free part of apodeme.

Fig. 6. "Annulated tube." × 370. *A.T.* "annulated tube"; *C.* III, 3rd coxa; C. IV, 4th coxa; *S.* sac; *T.* tendon.

Fig. 7. Gnathosoma. Adult female. × 220. *C.M.* corniculus of maxilla; *F.D.* fixed digit of mandible; *H.* hypopharynx; *M.* mandible; *M.D.* movable digit of mandible; *P.* maxillary palp; *S.M.I.A.* seta maxillaris interiores anteriores; *S.M.I.P.* seta maxillaris interiores posteriores; *S.M.E.* seta maxillaris exteriores.

Fig. 8. Gnathosoma. Adult male. × 250. *M.D.* movable digit of mandible; *S.* spermatophore carrier.

Fig. 9. Adult male. Ventral side. × 100. *A.P.* anal plate; *G.A.* genital aperture; *P.* peritreme; *T.* tritosternum; *S.M.G.P.* sternal-metasternal-genital plate.

EXPLANATION OF FIGURES 10–15.

Fig. 10. Larva. Ventral side. × 110. *A.P.* anal plate; *S.P.* sternal plate.

Fig. 11. Dorsal plate. Larva. × 110.

Fig. 12. Mandible. Larva. × 740.

Fig. 13. Protonymph. Ventral side. × 100. *A.P.* anal plate; *P.* peritreme; *S.P.* sternal plate.

Fig. 14. Dorsal plate. Protonymph. × 110.

Fig. 15. Peritreme. Protonymph. × 550. *S.* stigma.

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SOME GALL MIDGE SPECIES AND THEIR HOST PLANT RANGE

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(With Plate XXII)

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I. Introduction

Broadly speaking the taxonomist may take up one of two attitudes: either every insect sent in for identification can be considered as possibly new and undescribed until it is proved to be the same as one previously described, or else it can be presumed that the insect in question has already been described and must not be considered new until it can be proved to be different from all those previously described.

The latter method results in a tendency to classify temporarily as one several distinct species. From a biologist's point of view this may subsequently be disastrous if it is later shown that two or three species are really involved. All the previous literature concerning the bionomics and control in such a case becomes comparatively worthless and new investigations have to be commenced on each of the species.

The former method appears to be the more logical when it is realized that the number of known species is often but a fraction of those awaiting discovery, and that in many groups the present classification must of necessity be considered merely as the most convenient for the moment.

In the Cecidomyidae or gall midges, it is well known that it is sometimes exceedingly difficult to differentiate between closely allied species. This being so, workers in this group have been inclined to look upon midges reared from different host plants as separate species and then,

acting on this presumption, to find some minute differences of a morphological nature on which to base the description of the new midge. There is some excuse for this method. For instance, when only a few specimens are available, such minute differences appear real. (A vastly different state of affairs exists when one is dealing with hundreds of either sex, as in biological studies.) An advantage is that it keeps possibly distinct species apart until such time as it can be proved that they are identical. A name, apart from taxonomic considerations, is also an identification tag or key whereby it should be possible to look up all the available information concerning the insect in question.

However, as is illustrated below, this is precisely what so often does not result if each worker uses a different name (and therefore a different tag) for what in reality is one species. From an economic entomologist's point of view such a procedure can be very misleading, particularly if there is no subsequent literature concerning many of the species.

For example, in the literature concerning the species of the genus *Asphondylia*, 133 species have been described with host plant records. The plants recorded occur in forty families. All the 133 species are each limited to one family of host plants.[1] Among the *Asphondylia* species, 127 are limited to one genus of host plant, five species to two genera and one to three genera. Again, 103 species are limited to one species of host plant, fourteen to two species, four to three species, six to four species, three to five species, two to six species and one to twelve species; and in only a few instances has anything been published concerning these species since the original description and host plant record. If the question arose whether it would be considered a safe measure to introduce a certain *Asphondylia* species as a weed control, would there be any justification in the present state of knowledge in postulating that it was unlikely that the particular midge in question would limit its depredation to the only known host plant species?

Again, in the older literature there are references to *Contarinia medicaginis* Kieffer attacking the unopened blossom of *Medicago* spp. and *Lotus corniculatus*, and to *Contarinia loti* D.G. doing the same damage to *Lotus* spp. and also *Medicago* spp. and *Vicia* spp. These two species are comparatively common pests of seed production and numerous references to them occur in the literature. Yet it is only recently that the view that *Contarinia medicaginis* limits its attention to species of

[1] The only recorded instance of a gall midge living on plants belonging to two families is that of *Macrolabis corrugans* F.Lw. which is reported to live on certain Umbelliferous and Labiate species.

Medicago, and *Contarinia loti* confines itself to species of *Lotus*, has been accepted among those most intimately concerned.

The differences recorded in the descriptions of midge species are often so slight that it is wellnigh impossible even if the original specimens are examined to be sure that such differences are really specific and valid. The range of variation of such differences within a species is, with very few exceptions, almost unknown (Barnes, 1932).

Again, as, for example, *Rhabdophaga triandraperda* Barnes, *R. purpureaperda* Barnes and *R. justini* Barnes, some species are exceedingly difficult to separate on the adults alone. But the pupae, larvae and host plants taken together serve as a ready means of identification (Barnes, 1935 *a*). Among the species of the genus *Contarinia* living in grass-heads there appear few, if any, distinguishing morphological characters among the adults, pupae and larvae, and one has to depend on a knowledge of the host plants. This can only be obtained by biological experimentation.

It is evident therefore that what is needed, both for the grower and taxonomist, is that far more attention should be paid to acquiring biological information (for example, host plant range) and the emphasis taken away from the use of pure morphology alone as a means of separating species. The grower wants answers to such questions as: If he suffers from *C. loti* on his *Lotus*, can he safely grow Lucerne? If his *Salix triandra* is attacked by *Rhabdophaga triandraperda*, can he expect his *Salix purpurea* and *S. viminalis* to escape damage?

The present series of studies is based on such a motif. In the first instance the *Arabis* midge will receive consideration. Then will follow accounts of similar investigations on the chrysanthemum midge, the black-currant leaf midge and the hawthorn stem midge.

II. The *Arabis* midge

Historical

In the literature there appear three names of midges whose larvae cause terminal bud galls on *Arabis* species, namely, *Dasyneura alpestris* Kieffer (1909), *D. schneideri* Rübsaamen (1917) and *D. arabis* Barnes (Barnes & Theobald, 1927). A brief survey of the published information concerning these midges follows:

Dasyneura alpestris Kieffer. In 1909 Kieffer wrote:

"*Arabis alpina* L. et *hirsuta* Sc. Pousse terminale changée en une agglomération de feuilles élargies (Thomas, 1886). *Perrisia*? *alpestris* sp.n."

In this way Kieffer gave a name to the insect whose larvae had previously been noticed causing a terminal bud gall on *Arabis alpina* at Suldenthal in the Tyrol by Thomas (1886).

F. Loew (1888) mentioned a similar gall on *A. hirsuta* which he had found in Niederösterreich. Schlechtendal (1891), in his list of German gall formers, noted a midge doing this type of damage to *A. alpina* and *A. hirsuta*. Rübsaamen (1896), in his paper on Russian gall midges, stated that the midge causing the gall described by Thomas (1886) belonged to the genus *Dichelomyia*, but did not actually say that it had been found in Russia. de Meijere (1928) described the adults of a midge which he had found in Holland in terminal bud galls of *A. alpina* and retained the name *alpestris* Kieffer, calling the midge *Dasyneura alpestris* Kieffer. de Meijere at the same time suggested that this species was synonymous with *D. schneideri* Rübsaamen.

Dasyneura schneideri Rübsaamen. Rübsaamen (1917) described in detail, as *D. schneideri*, a midge whose larvae caused terminal bud galls on *Arabis albida* Stev.[1] At the same time he mentioned finding white larvae with "breastbones" slightly differing in shape from those of *Dasyneura schneideri* whose larvae were red. The adult midges belonging to this second species were not known to Rübsaamen who received *D. schneideri* from Dr Schneider-Orelli from Zurich.

Later the Deshusses (1936) reported *D. schneideri* from Switzerland doing extensive damage on *Arabis albida*. They stated that they had reared platygasterid parasites and gave information concerning the biology, and a description of the adult midge. There are excellent photographs of the gall in this paper. In addition, they mention that similar damage had been recorded on *A. alpina* in Switzerland by Moreillon (1916), Vogler (1906) and Perriraz (1909). Lastly these authors state that a memoir on the anatomy of *Dasyneura schneideri* will appear later.

Dasyneura arabis Barnes. In 1927 the present writer (Barnes & Theobald, 1927) described, as *D. arabis*, midges reared in England from larvae which answered the description of the second midge mentioned by Rübsaamen (1917), but whose adults were then unknown. Later Barnes (1935 *b*), in a study of fluctuations in insect populations dealing with *D. arabis*, wrote: "It seems quite possible that in the future it will be proved that these two species referring to *D. schneideri* and *D. arabis* are identical and that the differences observed between them fall within the range of variation." And later in the same paper he stated that *Arabis alpina* and *A. hirsuta* had not yet been tested as possible host plants of *Dasyneura arabis*. A species of *Lestodiplosis* was reported at that time as being predaceous on the larvae of *Dasyneura arabis* and *Misocyclops marchali* Kieffer was recorded as an internal parasite.

Present investigations

In the autumn of 1937 Dr H. Sachtleben gave the writer the opportunity of examining midges reared by Dr Pape, who had found the galls on *Arabis alpina* at Bad Soden-Allendorf and Kiel. These specimens answered exactly the description of *Dasyneura alpestris* Kieffer given by de Meijere in 1928.

At the same time inquiries revealed that *D. arabis* was present in

[1] This species is now considered a synonym of *Arabis caucasica* Willd.

England in Surrey, Middlesex, Hertfordshire and Cambridge. On the other hand, searches made for the galls were unproductive in Manchester, Oxford, Newcastle, Cornwall, Somerset, Devon and Caernarvonshire. Incidentally, in 1937 wherever the midge occurred it was found in great abundance. For instance, in almost every garden where *Arabis caucasica* was growing in Harpenden the galls were exceedingly obvious in September. On the other hand, in 1938 the galls were not at all plentiful.

The opportunity had arisen for attempting to determine biologically the status of the three species, *Dasyneura alpestris*, *D. schneideri* and *D. arabis*.

Accordingly, in September 1937, living material was obtained on *Arabis alpina* from Kiel, Germany and the Royal Botanic Gardens, Kew; on *A. alpina* var. *rosabella* from Kew; and on *A. caucasica* (*albida*) from the garden of Rothamsted Experimental Station, Harpenden, from Batford, Hertfordshire, and from Cambridge. Adult midges emerged in the outdoor insectary from the English material between 6 and 29 October and from the German material from 17 October to 18 November. These dates are much later than the latest previously[1] known (11 October) for *Dasyneura arabis* and probably partly account for the comparative scarceness of the midge in 1938. The larvae normally overwinter in the soil and in their cocoons on the plants. If most of the third, as in this case, or probably the fourth generation emerge and the resultant larvae cannot reach the necessary stage of development before adverse conditions set in, then there will be comparatively few individuals overwintering successfully. This has been observed previously in the leaf-curling pear midge (Barnes, 1935 *c*).

In addition to the extremely late emergences in October and November 1937, the midges started emerging in 1938 much sooner than in any year between 1929 and 1934. In the exceptionally early season of 1933 the first emergence occurred on 17 April instead of between 4 and 17 May in the other years. But in 1938 emergence started on 6 April. Frosty weather subsequently followed, which was another factor in decreasing the amount of damage due to this midge in 1938.

During the rearing of the *Dasyneura* midge material in 1938, besides breeding a few specimens of the *Lestodiplosis* sp. which had been previously reared, specimens of another midge were also bred. They belong to the genus *Macrolabis*. The breeding of this *Macrolabis* species probably provides the clue to the white larvae mentioned by Rübsaamen

[1] For details of the biology see Barnes (1935 *b*).

(1917) and their supposedly adult midges described by the present writer as *Dasyneura arabis* in 1927. For it was found that when dealing with large numbers of larvae in the fluctuation study (1935 *b*) the colour of the gall-forming larvae varied from white to red, and the suspicion was then aroused as to the validity of the separation of *D. arabis* from *D. schneideri*. Parasites were also bred from the English and German material and have been identified by Dr Ferrière as follows: Eulophidae. *Tetrastichus annulatus* Först. (Kiel on *Arabis alpina*, Kew on *A. alpina* var. *rosabella* and *A. alpina* and Harpenden on *A. caucasica*); *Omphale aetius* Walk. (Harpenden on *Arabis caucasica*). Platygasteridae. *Synopeas* sp.n.? (Kew on *Arabis alpina* and its variety *rosabella*); *Piestopleura catillus* Walk. (Kew on *Arabis alpina*).

Cross-mating experiments, 1938

In April 1938 newly emerged midges, chosen for their difference in previous host plant and locality, were given the opportunity of mating in small glass tubes standing in sand. All the crosses were successful, mating taking place in the normal time and lasting the usual period (Table I).

Table I. *Cross matings of* Arabis *midges reared on different* Arabis *spp. and from different localities*

	Male		Female	
	Sp. of *Arabis*	Source	Sp. of *Arabis*	Source
1.	*A. alpina* var. *rosabella*	Kew, Surrey	*A. alpina*	Kiel, Germany
2.	*A. alpina*	Kew, Surrey	*A. caucasica*	Batford, Herts.
3.	*A. alpina*	Kew, Surrey	*A. caucasica*	Cambridge
4.	*A. alpina*	Kew, Surrey	*A. caucasica*	Cambridge
5.	*A. alpina*	Kew, Surrey	*A. alpina*	Kiel, Germany
6.	*A. alpina*	Kew, Surrey	*A. alpina*	Kiel, Germany
7.	*A. caucasica*	Batford, Herts.	*A. alpina*	Kew, Surrey

In every case followed up, the cross matings were fertile and, in order to test the interchangeability of the host plant species, the females were allowed to oviposit on different species of *Arabis* (Table II).

Table II. *Breeding of the cross-mated midges on various* Arabis *spp.*

	Cross mating					
	Male		Female			
	Host plant sp.	Locality	Host plant sp.	Locality	Sp. of *Arabis*	Fertility
1.	*A. alpina*	Kew	*A. caucasica*	Batford	*A. aubrietioides*	Normal
2.	*A. alpina*	Kew	*A. caucasica*	Cambridge	*A. caucasica*	Normal
3.	*A. caucasica*	Batford	*A. alpina*	Kew	*A. alpina*	Normal
4.	*A. alpina* var. *rosabella*	Kew	*A. alpina*	Kiel	*A. caucasica*	Normal

It is of interest that in two cases where only one female was used, the offspring or G_1 showed a marked departure from the normal 50 : 50 sex ratio, thus supporting the previous finding that in *Dasyneura arabis* there are male-producing females and female-producing females in addition to females which produce families in which both sexes occur in approximately equal numbers (Barnes, 1931 *a*).

Natural infestation of Arabis *spp.*

In late September 1937 the *Arabis* spp. growing in the Royal Botanic Gardens, Kew, were inspected and it was noted that while some species were heavily infested, others near by had suffered no attack. It was evident that all the species of *Arabis* which looked like *A. caucasica* were attacked while other species, rather different in general appearance, showed no signs of damage or galls. Table III shows the species examined at Kew, those attacked by the midge, and the subgenus to which they belong. The writer is indebted to the courtesy of the Director and the authorities of the Herbarium, Royal Botanic Gardens, Kew, for the correct citations, names of the subgenera and the systematic notes concerning the species of *Arabis*. It can be seen from Table III that some species of the subgenus *Euarabis* were attacked by the midge (but not all the species of *Euarabis* which were present), and one species which may belong to the subgenus *Lomaspora*.

Immunity trials

Having observed a definite preference of the midges for certain species of *Arabis*, it was decided to subject the midges to immunity trials, i.e. give them the obligation to oviposit on a species of *Arabis* or else produce no offspring.

Accordingly, midges were introduced into cages containing only one of the following *Arabis* spp., obtained through the courtesy of the Director of the Royal Botanic Gardens, Kew: *A. alpina*, *A. aubrietioides*, *A. caucasica*, *A. carduchorum*, *A. hirsuta* var. *glabra*, *A. landaurii*, *A. procurrens* and *A. Scopoliana*. Five or six freshly emerged and impregnated females were placed in each cage in the trials carried out in April 1938, two pots of each species of *Arabis* being used, with the exception of *A. hirsuta* var. *glabra*, where only one plant was available. In the August trials three females of the midge were used in each cage and two plants of *A. Scopoliana* and *A. procurrens*, and one plant each of *A. landaurii*, *A. hirsuta* var. *glabra*, and *A. carduchorum* were subjected to trial.

After the insertion of the midges into the cages, observations were

made for the first few hours for signs of oviposition. In the *A. alpina*, *A. aubrietioides* and *A. caucasica* pots, oviposition was observed without exception to take place almost immediately. With all the other *Arabis*

Table III. Arabis *spp. at the Royal Botanic Gardens, Kew, indicating which were suffering from midge attack on* 28 *September* 1937

Arabis spp.	Subgenus	Midge galls present
A. alpina L.	*Euarabis*	×
A. alpina L. var. *rosabella*[1]	*Euarabis*	×
A. androsacea Fenzl.	*Pseudarabis*	–
A. aubrietioides Boiss.	*Euarabis*	×
A. bellidifolia Jacq.	*Euarabis*	–
A. blepharophylla Hook. & Arn.	*Euarabis*	–
A. brachycarpa Rupr.	*Lomaspora*	–
A. Breweri S.Wats.	*Turritis*	–
A. carduchorum Boiss.	*Drabopsis*	–
A. caucasica Willd. (*albida* Stev.)	*Euarabis*	×
A. Collinsii Fernald	*Turritis*	–
A. corymbiflora Vest.	*Turritella*	–
A. hirsuta Scop. var. *glabra* L. (Lange)[2]	*Turritella*	–
A. japonica Regel	See footnote [3]	–
A. Koehleri[4]	*Turritis*	–
A. landaurii[5]	*A. bellidifolia* × *Ferdinandi Coburgi*	–
A. Lyallii S.Wats.	*Turritis*	–
A. procurrens Waldst. & Kit.	*Pseudarabis*	–
A. Scopoliana Boiss.	*Drabina*	–
A. Stelleri DC. var. *rosea*	*Lomaspora*?[6]	×
A. tomentosa[7]	See footnote [7]	–

[1] ? horticultural form of *A. alpina*.

[2] *A. hirsuta* Scop. var. *glabra* L. (Lange) in *Fl. Dan.* **17**, t 2911, where the name *Turritis hirsuta* var. *glabra* L. is quoted in synonymy.

[3] *A. japonica* Regel et Herder (1863) (which may or may not be the same as *A. japonica* (A. Gray) A. Gray, 1858) was referred by its authors (*Gartenfl.* **12**, 309) to Sect. *Alomatium* DC. But they also stated that it stood "about midway between *A. alpina* L. and *A. brassiciformis* Wallr.", which are referred by O. E. Schulz (*Engl. Pflanzenfam.* ed. 2, **17**, 544, 1936) to Sect. *Brassicoturritis* O. E. Schulz. *A. japonica* (A. Gray) A. Gray is treated by Nakai (in *Bot. Mag. Tokio*, **32**, 241, 1918) as a var. *japonica* (A. Gray) Fr. Schmidt of *A. Stelleri* DC., a species placed by O. E. Schulz (*Engl. Pflanzenfam.* ed. 2, **17**, 545, 1936) in Sect. *Turritella* C. A. May.

On the basis of the original description, *A. japonica* Regel et Herder runs down in Schulz's key (*Engl. Pflanzenfam.* ed. 2, **17**, 543, 1936) to Sect. *Brassicoturritis*, but the sectional differences, depending on the erect or ascending position of the fruits and/or pedicels, seem very slight.

[4] Name not traced at Kew.

[5] A letter from the Director, Royal Botanic Gardens, includes the following statement: "*Arabis Landaueri* Sundermann in *Allg. Bot. Zeitschr.* **26–27**, 21 (1925), hybr.–Hort. We think this is what is meant by your name '*A. landaurii*'."

[6] *Stelleri* DC. is described in DC. *Syst.* **2**, 242 as most closely allied to *pendula*, which belongs to Sect. *Lomaspora*. A subsequent letter from the Director, Royal Botanic Gardens, includes the statement: "We cannot trace *Arabis Stelleri* var. *rosea*; we find, however, *A. Stelleri* DC., *Syst.* **2**, 242 (1821), and also *A. rosea* DC., *Syst.* **2**, 215."

[7] Name not traced at Kew.

spp., both in April and in August, attempts at oviposition, still less actual oviposition, were never observed.

Later, the plants were examined for galls. In each pot of *A. alpina*, *A. aubrietioides* and *A. caucasica* galls which ultimately gave rise to adult midges were observed. On the other hand, no galls and consequently no adult midges appeared on *A. carduchorum*, *A. hirsuta* var. *glabra*, *A. landaurii*, *A. procurrens* and *A. Scopoliana*.

Conclusions

In the light of these biological tests supported by the knowledge of the midges themselves, one is forced to the conclusion that there is only one real species involved in the formation of terminal leaf-bud galls on *Arabis*.

This species should be known as *Dasyneura alpestris* (Kieffer, 1909) de Meijere (1928), the adults of which were first described by de Meijere in 1928. The gall described by Kieffer is quite easily recognizable from the description and this type of gall is not caused on these plants by any other gall midge. The names *Dasyneura schneideri* Rübsaamen (1917) and *D. arabis* Barnes (1927) fall as synonyms.

D. alpestris (Kieffer) de Meijere will only live on certain *Arabis* spp. belonging to the subgenus *Euarabis* and possibly also *Lomaspora*, and will not attack species belonging to such other subgenera as *Pseudarabis*, *Turritis*, *Drabopsis*, *Drabina* and *Turritella*. The midge will not, however, attack all species of the subgenus *Euarabis*. It is shown to develop normally on *Arabis alpina*, *A. alpina* var. *rosabella*, *A. aubrietioides* and *A. caucasica*. Attempts to induce it to breed on five species in other subgenera failed.

In the literature the midge is recorded from *A. hirsuta*. This species itself has not been used in the experiments but its variety, *A. hirsuta* var. *glabra*, proved to be unattacked by the midge.

The second midge mentioned by Rübsaamen (1917) is probably the *Macrolabis* species which has been found living, and reared, as a commensal in the galls of *Dasyneura alpestris*.

III. The chrysanthemum midge

Briefly stated, the question to be considered is whether the chrysanthemum midge of commercial varieties is the same species as *Diarthronomyia hypogaea* F.Lw. of wild *Chrysanthemum* spp. It is important to note that in all the experiments described subsequently only midges

obtained from cultivated chrysanthemums have been used. No specimens from wild host plants have been available.

Distribution of the commercial chrysanthemum midge

United States of America. Felt (1915) identified as *Diarthronomyia* (*Rhopalomyia*) ***hypogaea*** **F.Lw.** (see next section) a midge which was causing serious damage to commercial chrysanthemums in a glasshouse during late March of that year at Adrian, Michigan. This is the first record of the chrysanthemum midge attacking commercial varieties, although according to Essig (1916) Californian florists stated that this pest had been present round San Francisco Bay for over 15 years. In 1915 it was definitely recorded from California and Oregon as well as from Michigan (Felt, 1916). By 1920 the midge had been reported from all the larger chrysanthemum-growing regions of the U.S.A., including California, Connecticut, Delaware, the District of Columbia, Georgia, Illinois, Indiana, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Ohio, Oregon, Pennsylvania, Rhode Island, South Dakota, Tennessee and Virginia (Weigel & Sanford, 1920).

Canada. This midge first appeared in Canada in 1915 at Ottawa where it occurred in one of the large greenhouses and, according to Gibson (1916), had doubtless been recently introduced with plants from the U.S.A. In August of the same year it was found on commercial chrysanthemums growing both outside and inside a greenhouse in Victoria, B.C. (Gibson, 1917; Treherne, 1916). Since that date it has become well established throughout the province of Ontario and has also been found in Quebec and Nova Scotia (Gibson & Ross, 1922).

Russia. Possibly the first European record of this midge on commercial varieties of chrysanthemum is that by Daniltchenko (1916). This writer, when dealing with chrysanthemums and their cultivation, stated that the collar is injured by this pest. Pending further information this record can only be considered as a warning to chrysanthemum growers. No exact information as to its status in Russia is available, but Miss Dombrowski has informed the writer (*in litt.* March 1937) that she has found *Diarthronomyia hypogaea* only on wild *Matricaria inodora* L. and *M. chamomilla* L.

England. The first outbreak was discovered in England in 1927 in the Lea Valley (Speyer, 1927 *a*, *b*, 1928) and two subsidiary infestations were noted in Bedfordshire and in Norfolk (M. of A. 1928). In the autumn of 1928 (Miles, 1929) the midge was found in a commercial glasshouse in South Lancashire. Two infestations were known to persist in the country in the autumn of 1930 (M. of A. 1933), but in 1936 (M. of A. 1936) the Ministry of Agriculture was in a position to state that the eradication campaign had been successful as no occurrence of the pest had been found since 1930.

The second outbreak in England occurred in 1936 and the midge was discovered in several nurseries in Sussex, with four isolated infestations in Essex, Gloucestershire and Glamorganshire.

Up to December 1938 the midge was still present in Sussex and Essex.

Both these outbreaks were traced to chrysanthemum varieties imported from the U.S.A.

Ireland. Mr R. Chamberlain has recently informed the writer that the midge was found on 31 December 1937 at Belfast. In this case the outbreak was traced to an infested area in England.

Denmark. The midge was first recorded attacking commercial chrysanthemums in Denmark in 1934. P. Bovien (1935) found galls in the open and in greenhouses during the winter. The same writer (1937) reported its occurrence in 1936 in two new localities including the island of Funen near Copenhagen.

Finland. The midge first appeared in 1936 in Finland and has become established in a few glasshouses in the vicinity of Helsinki and Turku. In one case it was evidently imported from Germany and in another from Sweden. The writer is indebted to Mr Niilo A. Vappula for this information and to Mr Y. Hukkinen for specimens.

Sweden. Commercial chrysanthemums were found infested by this midge in the Stockholm area early in November 1936 (Statens Växtskyddsanstalt, 1936) and it is stated that probably it was imported from Denmark. The midge was found in at least twelve places from Skåne northwards to Southern Norrland, and in two instances it had been seen by growers in 1935 but not reported. Later, Ahlberg (1938) stated that irregular watering and high humidity are partly responsible for the depressions found near the actual galls.

France. Suire (1935) mentions *Diarthronomyia hypogaea* Lw. in his list of insects attacking chrysanthemums in France. Prof. A. Balachowsky (*in litt.* 10 October 1938) stated that nothing has been published recently in France concerning this midge attacking autumn chrysanthemums.

Germany. Dr Hans Sachtleben of the Deutsches Entomologisches Institut has informed the writer (*in litt.* 8 September 1938) that the occurrence of the midge on cultivated chrysanthemums in Germany was till then slight and only local.

From the above summary of the midge's distribution on commercial chrysanthemums, it can be seen that it first occurred as a pest in the United States of America. By 1916, only one year after it was first recorded, besides being established in California, Oregon and Michigan, it had already been reported from British Columbia and Ottawa in Canada. The first definite occurrence of the midge as a pest in Europe was in England in 1927, and a further outbreak began in 1936. Other outbreaks have occurred in Europe in recent years. It appeared in Denmark first in 1934 and apparently is still present. In 1935 it arrived in Sweden and in the next year reached Finland.

Identification

When the chrysanthemum midge was first discovered in the United States of America, Felt (1915) identified it as *Diarthronomyia* (*Rhopalomyia*) *hypogaea* F.Lw.

This species had been described by F. Loew (1885) from specimens of subterranean galls, two males and pupae found on *Chrysanthemum atratum* Jacq. in Central Europe (Raxalpa) by E. Berroyer in 1875. Lemée (1902) recorded this midge forming galls on the flowers, leaf buds and stems of *C. Leucanthemum* L. in France, and Baldrati (1900) reported and figured it from the same plant in Italy. Lemée (1902) also recorded

it from *C. corymbosum* L. and a midge, possibly the same species, on *C. japonicum* Thunb.[1] Cotte (1912) recorded it from *C. Myconis* L. and figured the gall (Houard, 1913).

During recent years doubts have been growing as to whether the midge of commercial chrysanthemums is *Diarthronomyia hypogaea* F.Lw. (1885). The galls figured by Baldrati and by Cotte bear little resemblance to those caused by the midge on commercial chrysanthemums and, moreover, the commercial chrysanthemum midge has not been known to spread to wild *Chrysanthemum* spp. For example, Weigel & Sanford (1920) wrote: "Although several attempts have been made to infest the Shasta daisy and the common field daisy, *C. Leucanthemum*, it has not been possible to get the ovipositing female to lay eggs on them." Again, Hamilton (1924) stated that experiments were carried out to see if the midge would reproduce on any of the chrysanthemums other than the cultivated greenhouse varieties. *C. Leucanthemum* and its cultivated variety the Shasta daisy (*C. maximum*) were used but the females did not oviposit on them. An infestation was secured on the annual or summer varieties (mixed seed and probably *C. coronarium*). Eggs were laid on four of the plants and galls subsequently formed on all four. Distinct galls were not formed but the leaves were curled and twisted while the stems and buds were deformed. No adult midges however developed.

Similarly, Miles (1929) failed to find signs of its presence on wild ox-eye daisy in various parts of south Lancashire and north Cheshire, although, in the autumn of 1928, the chrysanthemum midge was occurring on commercial plants in south Lancashire. Efforts on the part of the writer to find the midge on wild ox-eye daisy from 1927 to date have also failed. Mr N. A. Vappula (*in litt.* 1 September 1938), in reply to the question whether it had been found on wild chrysanthemums in Finland, wrote that up to the present it had been found only on glasshouse chrysanthemums. Similarly Mr P. Bovien (*in litt.* 23 July 1938) stated that he had not found it on *C. Leucanthemum* in Denmark.

The following quotation from an unsigned article in the *Journal of the Ministry of Agriculture* (Anon. 1937) summarizes the current opinion held in England: "When the midge (*Diarthronomyia* sp.) was first discovered in America it was recorded under the name of a European species (*Diarthronomyia hypogaea* F.Lw.), known since 1885 as attacking the roots of various kinds of ox-eye daisy. This original identification, however, seems to have been accepted in Europe without further investigation and without experimental evidence to show whether the daisy

[1] In *Index Kewensis* this species is considered to be *C. indicum* L.

midge will in fact attack greenhouse chrysanthemums. In view of the difference in habit between the two insects, it is not improbable that they may prove to be distinct species. However this may be, it is clear from the horticultural point of view the chrysanthemum midge must be regarded as a foreign pest...."

Present investigations

Fruitless efforts have been made to rediscover *Diarthronomyia hypogaea* F.Lw., reported by Bagnall & Harrison (1921) on *Chrysanthemum Leucanthemum* at Babbacombe and Sidmouth, Devon, both in the latter locality and elsewhere in England. Similarly, attempts to obtain material from wild chrysanthemums on the continent of Europe have proved of no avail.

Material of the chrysanthemum midge on commercial chrysanthemums, however, has been supplied by Mr A. S. Buckhurst of the Ministry of Agriculture from the Worthing area in February 1937 and from south Wales in December 1937.[1]

The Ministry of Agriculture having granted the writer a licence to keep specimens of the chrysanthemum midge, an attempt has been made to find the host range of this midge and also to study its life history in this country. A later report will deal with the latter. The following section gives the results of attempts to induce the midge to breed on different species of *Chrysanthemum* and other allied plants.

Host range

(i) *Species and varieties of* Chrysanthemum.

Commercial varieties of autumn chrysanthemum. The following varieties were used for breeding the midge both in the laboratory and in an unheated open greenhouse: Crimson Circle, September White, September Pink, Phoenix, Mrs Arkwright, "Rose Pink" and Cranford Yellow. In all cases, even in pots in which only one female midge had been placed, oviposition took place followed by the development of galls and, with the exception of one pot in which the plant died, adult midges subsequently emerged. These breedings may be considered as the controls.

C. Leucanthemum L. (ox-eye or dog daisy). In January 1938, 14, 11, 9, 8, 11 and 9 females were introduced into six pots containing *C. Leucanthemum* plants in the laboratory. One female was observed ovipositing on a stem just behind a leaf petiole.

[1] Parasites were bred from both samples. Those which were reared from the Worthing area were kindly identified by Dr C. Ferrière as *Eutelus diffinis* Walker.

Larvae subsequently hatched but no galls were formed. In late March and early April two more attempts to induce breeding on this plant using 3 and 5 female midges respectively were made, this time the plants being kept in the greenhouse. No oviposition was observed and no galls or adults developed. In May, 4 females were placed in a cage over an ox-eye daisy plant in the laboratory and 7 females were also put in each of two similar pots in the greenhouse. In one of the cages in the greenhouse oviposition was observed. This took place in the open blossom among the yellow disk florets, between the involucral bracts of open flower-heads and in the axils of the leaves on the flower stems. However, again no galls or adults developed. In September two more pots were set up, each with 8 female midges. No oviposition, gall formation or adults were subsequently observed.

Thus, having used 104 females on 17 different plants in January, March–April, May and September both in the laboratory and in the greenhouse, oviposition was observed on only two plants, and in no case did galls or adults develop.

C. Parthenium Bernh. In January two pots of this plant were set up in the laboratory and 21 and 12 female midges respectively were used. Oviposition readily took place in each pot in the terminal leaf buds and on the stems of the plant. Later small pustules developed on the leaves and corrugations on the stems. Young larvae were dissected out of both the pustules and the corrugations. No adults developed. In April, 3 females were placed on a plant in the greenhouse. No oviposition was observed, no gall formation resulted and no adults emerged. In May, four pots of this plant were set up in the laboratory using 4, 4, 5 and 6 female midges respectively and one pot in the laboratory using 4 females. In one pot in the greenhouse and in the pot in the laboratory, oviposition was observed in the terminal leaf buds but no galls or adults developed. In September two more plants were used in the greenhouse, 7 and 8 female midges being placed on them. No egg-laying was observed and no galls or adults resulted.

Thus, having used 74 females on 10 different plants in January, April, May and September both in the laboratory and in the greenhouse, oviposition was observed to take place on four plants, galls were formed on two plants, but adults were never obtained.

C. indicum L. Plants of this species were kindly supplied by the Director of the Royal Botanic Gardens, Kew. Two plants were subjected to trial in January in the laboratory, 1 and 7 female midges being used. In each case, egg-laying took place immediately on the young leaves and buds. Unfortunately, the plants died very quickly before galls had time to develop. In April two more plants were used in the greenhouse, 1 female midge being placed on each plant. Oviposition at once took place, galls developed on the leaves and adult midges of generation 1 duly emerged. In May, 2 females were placed on a further plant in the laboratory, egg-laying took place but again the plant died prematurely. At the same time two further plants were used in the greenhouse and 2 and 1 female midges were used respectively. Egg-laying took place in each pot, galls developed on the leaves (Pl. XXII, fig. 1) and leaf petioles and adults emerged successfully.

Thus having used 15 females on 7 different plants in January, April and May both in the laboratory and in the greenhouse, oviposition took place immediately on all seven plants, while galls formed on and adults emerged from four plants. Three plants used in the laboratory died before the galls had time to develop.

C. cinerariaefolium Vis. Plants of this species were obtained from the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden. In January two plants were subjected to trial in the laboratory using 10 and 5 females respectively. Eggs were found in the uncurled leaf segments at the extremities of the leaves on one plant. No galls or adult midges developed. In May, one further plant was used in the laboratory and 4 female midges were placed on it. Egg-laying at once took place. The females appeared very keen to lay eggs in the parts of the leaves which were uncurled, while 1 female laid in an unopened flower bud. No galls or adults however developed. In May, also, two plants were tested in the greenhouse using 2 and 4 female midges. Egg-laying on one plant was observed, again in the uncurled portions of the leaves, but no galls or adults developed.

Thus, having used 25 females on 5 different plants in January and May both in the laboratory and in the greenhouse, oviposition was observed on three plants, but in no case did galls or adults develop.

C. coccineum Willd. (*roseum* Adam.). One plant of this species, obtained from a florist, was subjected to trial in the greenhouse in May using 4 female midges. Egg-laying took place in the opening flower-heads and among the involucral bracts. No galls or adults developed.

C. rubellum Sealy (*erubescens* Hort.). Plants of this species were obtained from the Royal Horticultural Society's Gardens, Wisley, through the kindness of Mr G. Fox Wilson. In May, two plants were subjected to trial in the greenhouse using 1 female midge per plant. In both cases oviposition took place at once on the extremities of the shoots, galls subsequently developed on the leaves (Pl. XXII, fig. 2), and adult midges duly emerged.

Two other plants of what apparently is this species were obtained from Miss A. Dixon, who had received the plants from Mr H. L. Jones. He in turn had received them from the Rev. D. A. Jones, Vicar of Rhuddlan, who obtained the plants from north Russia. 5 or 6 female midges were placed on each plant in the greenhouse in May. Oviposition took place immediately among the buds or shoots, galls subsequently developed and later adult midges emerged.

Thus, using 13 female midges on 4 different plants, from two localities, in the greenhouse during May, egg-laying was observed on each plant and later galls and adults successfully developed on each.

C. maximum Raymond (Shasta daisy). Plants of this species were obtained from the Royal Horticultural Society's Gardens, Wisley, again through the kindness of Mr G. Fox Wilson. One plant was tested during May in the laboratory and three in the same month in the greenhouse, using 5, 2, 4 and 3 female midges respectively.

Egg-laying was observed on the buds and young shoots on the plant in the laboratory and on one plant in the greenhouse. No galls or adult midges developed.

C. uliginosa Pers. One plant of this species was under trial in the greenhouse during May, 7 female midges being used. Oviposition took place immediately, but no galls or adults developed.

C. coronarium L. Two plants were subjected to trial during May in the greenhouse, 4 and 3 female midges being used. Oviposition took place among the young leaves just below the flower buds on both plants. No galls or adults developed.

C. frutescens Thunb. Two plants of this species, using 9 and 7 female midges respectively, were under trial during May in the greenhouse. Egg-laying was observed on each plant but again no galls or adult midges developed.

C. Zawadskii Herbich. Two plants of this species were obtained from the Royal Horticultural Society's Gardens at Wisley, through Mr G. Fox Wilson, and were tested during August in the greenhouse, using 5 and 10 female midges. No egg-laying was observed and no galls or adults developed.

C. azaleanum Hort.[1] A plant of this species was obtained from a local florist and 2 female midges were put on it in May in the greenhouse. Egg-laying took place at once on the developing shoots and subsequently typical galls and adults developed.

C. Korean Apollo. One plant obtained from a local florist was subjected to trial in May in the greenhouse. Oviposition took place immediately and typical galls and adults duly developed.

A summary of these results on different species and varieties of *Chrysanthemum* is given in Table IV. The chrysanthemum midge of commercial chrysanthemums was reared successfully on *C. indicum*, *C. rubellum*, *C. azaleanum* and *C.* Korean Apollo. Oviposition, however, was observed on all thirteen species and varieties used with the exception of *C. Zawadskii*, and it is possible that eggs may have been laid on this species also and remained unobserved. On the other hand, galls or malformations occurred only on *C. Parthenium* in addition to the species on which the midge bred successfully. In no case did galls develop on the roots, but only on the leaves and stems.

(ii) *Other plants*

Matricaria inodora (corn feverfew). Two plants were tested in the laboratory during January, 11 females being placed on each plant. Egg-laying took place in the open flower-heads on each plant, but no galls or adult midges developed. Another

[1] A letter from the Director of the Royal Botanic Gardens contains the following remarks after stating that they could find no trace of the name *C. azaleanum*. "I find, however, that there is a plant in the Trade which is listed under the name *Chrysanthemum indicum* var. *azaleoides*, and this appears to be the same plant as the one you sent us under the name *azaleanum*. Your plant is not a true variety of *indicum* though it may have some *indicum* blood in its pedigree; it does not appear to be anything more than one of the varieties or forms of the garden Chrysanthemums, which, of course, have some *indicum* blood in them. A specific Latin epithet should, of course, not be given to a horticultural variety as it leads to much confusion and misunderstanding."

Table IV. *Summary of host range experiments on chrysanthemum midge* 1938. *I. Species and varieties of* Chrysanthemum

Plant	No. of females used	No. of plants	Egg-laying on *x* plants	Galls formed on *x* plants	Adults obtained in *x* pots
C. Leucanthemum L.	104	17	2	Nil	Nil
C. Parthenium Bernh.	74	10	4	2	Nil
C. indicum L.	15	7	7	4	4*
C. cinerariaefolium Vis.	25	5	3	Nil	Nil
C. coccineum Willd. (*roseum* Adam.)	4	1	1	Nil	Nil
C. rubellum Sealy (*erubescens* Hort.)	13	4	4	4	4
C. maximum Raymond	14	4	2	Nil	Nil
C. uliginosa Pers.	7	1	1	Nil	Nil
C. coronarium L.	7	2	2	Nil	Nil
C. frutescens Thunb.	16	2	2	Nil	Nil
C. Zawadskii Herbich.	15	2	Nil	Nil	Nil
C. azaleanum Hort.†	2	1	1	1	1
C. Korean Apollo	3	1	1	1	1
Autumn chrysanthemums	Hundreds	Many	100%	100%	All save one

* Three plants died before gall formation. † See footnote on p. 333.

plant was tested during March and early April in the greenhouse using 5 female midges; oviposition was not observed and no galls or adults developed. In May, three further plants were subjected to trial; one in the laboratory and two in the greenhouse using 6, 2 and 4 female midges. Again no oviposition was observed and no galls or adults developed.

Thus using 39 females on six different plants both in the laboratory and in the greenhouse in January, March–April and in May, oviposition was observed on two plants but in no case did galls or adults develop.

This is interesting in view of Miss H. Dombrowski's record of finding *Diarthronomyia hypogaea* on *Matricaria inodora* and *M. chamomilla* (Dombrowski, 1936).

Anthemis nobilis (common chamomile). Plants of this and the next species were obtained from the Director of the St Ives Research Station, Bingley. One plant, using 7 female midges, was subjected to trial during January in the laboratory. Oviposition was observed, but no galls or adults developed. In May one other plant was used in the laboratory with 4 female midges and three plants, involving in each case 4 female midges, were used in the greenhouse. In no case was egg-laying seen, nor did galls or adults develop.

Anthemis Cotula (stinking mayweed or chamomile). One plant using 7 females was tested during January in the laboratory. Eggs were laid on the growing points. No galls or adults developed.

Achillea Millefolium (yarrow). Oviposition immediately occurred among the developing leaf buds of one plant which was given a trial using 11 females during January in the laboratory. No galls or adults developed.

Senecio vulgaris (groundsel). Oviposition took place on the stems of one plant which was given a trial using 11 females during January in the laboratory. No galls

or adults developed. This plant was in the same pot as the yarrow and the midges, having the choice of laying on either, oviposited on both.

In addition, one plant each of *Fragaria* sp. (strawberry), *Plantago lanceolata* (ribwort plantain), *Ranunculus repens* (creeping buttercup) and *Cheiranthus* sp. (wallflower) were tested all together in one pot in the laboratory, using 8 females. No signs of oviposition were observed, no galls were formed and no adults developed.

A test of the susceptibility of an *Artemisia* sp., on which a *Diarthronomyia* species of midge[1] was found breeding in Essex by Mr D. C. Thomas, was also made in July in the laboratory. Twenty-one females of the chrysanthemum midge were given the opportunity of ovipositing, but not one was observed to do so. No galls developed.

A summary of these results of experiments using plants other than *Chrysanthemum* spp. is given in Table V.

Table V. *Summary of host range experiments on chrysanthemum midge* 1938. II. *Plants other than* Chrysanthemum *spp.*

Plant	No. of females used	No. of plants	Egg-laying on x plants	Galls formed on x plants	Adults obtained in x pots
Matricaria inodora	39	6	2	Nil	Nil
Anthemis nobilis	23	5	1	Nil	Nil
Anthemis Cotula	7	1	1	Nil	Nil
Achillea Millefolium	11	1	1	Nil	Nil
Senecio vulgaris		1	1	Nil	Nil
Fragaria sp.	8	1	Nil	Nil	Nil
Ranunculus repens		1	Nil	Nil	Nil
Plantago lanceolata		1	Nil	Nil	Nil
Cheiranthus sp.		1	Nil	Nil	Nil
Artemisia sp.	21	1	Nil	Nil	Nil

Discussion

The experiments described show that the chrysanthemum midge will oviposit on a number of different species of *Chrysanthemum* and other plants belonging to the same family in addition to commercial chrysanthemums. There were, however, always commercial chrysanthemums near the experimental pots and if the presence (smell) of commercial chrysanthemums is a stimulus, this might account for the oviposition observed.

On one species only of *Chrysanthemum* did galls form followed by no adult development. It would appear, therefore, that in most cases the very young larvae could not establish themselves on the plants.

About 400 female midges were used in these experiments so that it may rightly be maintained that, if only a small fraction of a population of

[1] The species of *Diarthronomyia*, referred to above, which was found breeding on *Artemisia* was given the opportunity of ovipositing on September White variety of commercial chrysanthemum. Thirty-three females were used in the insectary. A few eggs were laid on the developing shoots but no galls developed and no adults emerged.

chrysanthemum midges can survive on plants other than commercial varieties of chrysanthemum, these tests are not conclusive. Further, it has not been possible to make experiments with *Diarthronomyia hypogaea* F.Lw. from wild *Chrysanthemum* spp.

A knowledge of gall midges as a group, however, supports the view that it is not impossible for species to be differentiated on such biological criteria, and the experiments lend support to the view that the chrysanthemum midge of commercial chrysanthemums is at least a distinct biological species from *Diarthronomyia hypogaea* of wild *Chrysanthemum* spp.

Summary and conclusion

The chrysanthemum midge of commercial chrysanthemums was found to breed on *Chrysanthemum indicum* (one of the species from which the autumn commercial chrysanthemums have been derived), *C. rubellum*, *C. azaleanum* (=*C. indicum* var. *azaleoides*) and *C.* Korean Apollo.

Oviposition took place and galls developed on *C. Parthenium*, but no adults developed.

Oviposition took place also on *C. Leucanthemum*, *C. cinerariaefolium*, *C. coccineum*, *C. maximum*, *C. uliginosa*, *C. coronarium* and *C. frutescens*, but no galls were formed or adults developed.

Oviposition was not observed on *C. Zawadskii*; no galls or adults developed.

Oviposition also took place on *Matricaria inodora*, *Anthemis nobilis*, *A. Cotula*, *Achillea Millefolium* and *Senecio vulgaris*. No galls or adults developed on these plants.

Other plants tested included *Fragaria* sp., *Ranunculus repens*, *Plantago lanceolata*, *Cheiranthus* sp. and *Artemisia* sp. No oviposition or gall formation occurred, and no adults developed.

Another species of *Diarthronomyia* which breeds on *Artemisia* sp. laid eggs on September White variety of chrysanthemum but no galls or adults developed.

These experiments support the view that the midge of commercial chrysanthemums is distinct from *Diarthronomyia hypogaea* of wild *Chrysanthemum* spp.

IV. The black-currant leaf midge

Introductory

In 1891 Rübsaamen (1891) described this midge, *Dasyneura tetensi* Rübsaamen, which he had bred from pale yellow larvae found in folded and twisted leaves on the terminal shoots of black currant (*Ribes nigrum*).

Later, the same authority (1912) stated that, having reared midges from similarly contorted leaves of gooseberry (*R. Grossularia*), he was convinced that they were the same species, namely *Dasyneura tetensi* Rübsaamen. With the solitary exception of a record by Bagnall & Harrison (1918) stating that they had found *D. tetensi* on gooseberry as well as on black currant in Durham, all other published accounts of *D. tetensi* refer to black currant as being the host plant.

On the other hand, a much less well known midge, *D. ribicola* Kieffer, has been recorded from folded leaves of gooseberry (Kieffer, 1909), but the adults of this species have not yet been described.

A similar crinkling and folding of the leaves is made by the larvae of *D. plicatrix* H.Lw. on various species of wild and cultivated *Rubus*, including blackberry (*R. fructicosus*), American blackberry, dewberry (*R. caesius*) and raspberry (*R. Idaeus*).

Life cycle on black currant

In some years in this country three generations can occur in the course of twelve months. The larvae overwinter in the soil and the first flight of midges occurs in April, May and early June. The larvae appear in the terminal leaves in late May and June. The second flight of adults takes place in late June and early July and the third in late July and August. The female midges lay their eggs on the growing tip and the lateral buds. As a consequence the leaves, instead of unfolding normally, become more and more folded and crinkled. Pupation takes place in the soil, and when the crinkled leaves turn black the larvae, having become full grown, are on the point of leaving the plant for the soil.

Host plant and cross-mating experiments

In 1930 (Barnes, 1931 *b*) tests were made to discover whether midges reared from black currant collected in Kent would oviposit on black currant, red currant, gooseberry and blackberry. Cages were set up containing black currant and wild blackberry shoots; blackberry shoots alone; black currant, red currant and gooseberry shoots; and black currant shoots alone. In each cage containing black currant the midges were seen to lay very readily on the black currant, but in no case was oviposition observed on blackberry, red currant or gooseberry.

In 1937 the writer repeated his 1930 experiments, this time on growing plants of black currant, gooseberry and wild blackberry, each in separate cages. There was thus no choice left for the midges. Midges reared from larvae obtained on black currant in Kent were used. Ovi-

position took place immediately on both plants of black currant, but no egg-laying was seen on either the gooseberry or blackberry.

Attempts were also made in 1937 to cross-mate midges (*Dasyneura tetensi*) reared from black currant with midges (*D. plicatrix*) reared from blackberry. When virgin females of *D. tetensi* were placed in tubes with males of *D. tetensi*, their ovipositors were extruded to their full extent and were waved about. The males were excited: the antennae vibrated, their wings were set in motion and the midges rushed up and down the tubes until they reached a female when mating took place.

When males of *D. plicatrix* were placed in tubes containing virgin *D. tetensi* females, the latter did not extrude their ovipositors and the males did not become excited and merely ignored the females.

When males of *D. plicatrix* and *D. tetensi* were placed in tubes containing virgin females *D. tetensi*, the last named extruded their ovipositors and the male *D. tetensi* became excited, whereas the male *D. plicatrix* remained unmoved.

In 1938 further attempts were made to induce midges, reared from larvae obtained on black currant in Kent and Hampshire, to oviposit on gooseberry. Three bushes each of gooseberry and black currant were used. Infected leaves of black currant containing larvae were placed under each of the bushes which were grown in pots and covered with muslin cages. During the period the midges were emerging observations were made frequently. Egg-laying was seen on several occasions on each of the black-currant bushes, but no oviposition was seen at all on any of the gooseberry bushes.

In addition, a few females were placed in a cage containing flowering currant (*Ribes sanguineum*) shoots. No egg-laying was observed. It is hoped to repeat this particular trial at some future date.

Discussion and conclusions

There are three very closely allied midges whose larvae cause the leaves of *Rubus* spp., *Ribes nigrum*, and *R. Grossularia* to remain folded and become twisted and crinkled. They are *Dasyneura plicatrix* H.Lw. on blackberry, *D. tetensi* Rübsaamen on black currant, and *D. ribicola* Kieffer on gooseberry. The adults of *D. ribicola* have not yet been described and the writer has had no opportunity of using material of this species.

D. plicatrix and *D. tetensi* are, morphologically, exceedingly difficult to separate but experiments made in 1937 have shown that these species will not cross-mate. *D. plicatrix* is recorded as living on *Rubus* spp. and

it has been reared on several occasions by the writer on plants of this genus. *Dasyneura tetensi* is recorded from Germany, Finland and the counties of Kent, Hampshire, Lancashire, Essex and Durham in this country. With the exception of one German and one English record of the larvae being found on gooseberry, all the records are from black currant. Confirmation of this is to be found in specimens in the writer's collection from Finland, Kent, Essex and Lancashire, in each case from black currant.

Whereas it has been possible in experiments to breed successfully *D. tetensi* on black currant on every occasion attempted, not once has the writer been able to observe oviposition on gooseberry, red currant, flowering currant or blackberry. On these latter plants in no case did the typical contortion of the leaves appear nor did adult midges develop. These experiments were carried out in three different years (1930, 1937 and 1938) and in each year fresh wild material from black currant in Kent was used. In 1938 material from Hampshire was also used.

In conclusion, therefore, it appears unlikely that *D. tetensi* Rübsaamen from black currant can breed on gooseberry, and it would seem best to regard *D. tetensi* as restricted to black currant and the two records of this species being found on gooseberry as probably referring to *D. ribicola* Kieffer, which was originally found in folded leaves of gooseberry. The question, however, cannot be finally settled until specimens of *D. ribicola* on gooseberry have been found, experiments made to induce them to oviposit and breed on black currant and attempts made to cross-mate midges from gooseberry with others from black currant.

V. The hawthorn stem midge

Introductory

In August 1937 Mr H. C. F. Newton noticed very common and widespread damage to hawthorn hedges in Shropshire, Staffordshire and Warwickshire. Young branches were being killed and became conspicuous owing to the withered leaves turning brown. Gall-midge larvae were present in the stems and death of the branches occurred above the points attacked. The bark at the infested areas flaked off easily, revealing clusters of pink larvae. The colour of the larvae was typical of the genus *Thomasiniana* as represented by *T. oculiperda* Rübsaamen, the red bud borer, and *T. theobaldi* Barnes, the raspberry cane midge. Material was received on 13 August and one male and one female emerged on 8 and

22 September respectively. These provided confirmation of the tentative identification that the damage was caused by a species of *Thomasiniana*.

Dr F. W. Edwards informs the writer that some years ago he noticed similar dying of shoots with midge larvae present on hawthorn hedges in Hertfordshire. A single larva of this species was found at Kinsbourne Green, Harpenden in September 1937 (Cecid. 3185).

The only references found in the literature to midges infesting hawthorn stems include one by Rudow (1875), who stated that attacked branches were swollen, the epidermis becoming brown and flaking off easily. Bergenstamm & Loew (1877) referred to Rudow's note, and Schlechtendal (1891) mentioned it again. No name was suggested for the insect causing this malformation, but it appears possible that these authors were dealing with the midge under discussion.

Life history

There appear to be two generations during the year. As has already been stated two adult midges emerged at Harpenden in September 1937 from material collected in Staffordshire on 12 August. Emergence started again on 26 May 1938 and continued until 13 July, the peak of emergence being about 11 June.

Midges were ovipositing in experiments from 3 to 15 June and the second generation of adults appeared on the wing from 1 to 14 August. The time of development for the summer generation was 56–62 days.

Pupation takes place in the soil. Emergence of the midges was noticed to occur regularly between 4.30 and 8.30 p.m. (British summer time). Mating lasted just over 2 min. and the fertilized females lived about 5 days, though in one case a female was observed still ovipositing on the eighth day after emergence. Normally oviposition by a single female is spread over several days.

Egg-laying was only observed to take place in artificial and natural slits in the bark of hawthorn stems; where the stems were undamaged no oviposition occurred. Egg-laying started about 7 p.m. and continued at least until dark (in early June). During the earlier part of the day the midges remained settled on the stems of hawthorn and the sides of the cages.

In two breeding experiments in which 3 females and 1 female were used, the number and sexes of the progeny were 3 males and 35 females and 19 females only respectively; an indication that unisexual progeny occur in this species. The sex ratio of bulk rearing was 46 : 54, as would be expected.

Only two individual parasites were reared, while 252 midges emerged from wild material.

Similarity between the red bud borer, raspberry cane midge and the hawthorn stem midge

Morphologically, the red bud borer (*T. oculiperda* Rübsaamen), the raspberry cane midge (*T. theobaldi* Barnes) and the hawthorn stem midge are very similar. On one occasion a male *T. oculiperda* mated with a female *T. theobaldi*, but this has never been repeated. The colour of the larvae is also a peculiar pink in each species.

Biologically, the three midges, so far as is known, are very similar. The adults all rest in a characteristically flattened attitude. The females of *T. oculiperda* oviposit under grafts and, where no grafts are available, in slits made artificially in the stems of rose trees; those of *T. theobaldi* lay under the broken skin of raspberry canes; while those of the hawthorn stem midge lay their eggs in slits artificially made in the branches of hawthorn as well as in any naturally occurring breaks in the bark.

Emergence of the adults of *T. oculiperda* took place regularly in the evenings from about 4.30 to 8.30 p.m. (British summer time), as did also those of the hawthorn stem midge. Egg-laying was observed in both species to take place in the evenings, at any rate up to dark. No information on these points is available concerning *T. theobaldi*.

In mass breedings the sex ratio of all three midges is roughly 50 : 50, but slightly in favour of the females. *T. oculiperda* was shown (Barnes, 1931*b*) to reproduce sexually by means of unisexual families, and there is now evidence which indicates that the hawthorn stem midge does the same. Nothing is known in this respect concerning *T. theobaldi*.

T. oculiperda has three generations a year. In 1930 the first flight was on the wing during the latter part of May and nearly all June, the second in July and early August, and the third in late August and September. *T. theobaldi* so far as is known has one generation a year: the adults being on the wing—in 1929 and 1930 —in the latter part of May, throughout June and the early part of July. The hawthorn stem midge has two generations a year. The first flight in 1938 appearing from late May, throughout June and in early July, while the second was on the wing during the first fortnight of August. A few emergences also took place in September in 1937.

All the above information has been derived from experiments carried out by the writer at the Rothamsted Experimental Station.

Host plant experiments

In view of the similarity between the biology of these three midges and the difficulty in separating the adults on morphological grounds, it was decided to carry out experiments concerning the host plant range.

Previously (Barnes, 1931 *b*) unsuccessful attempts were made to induce *T. oculiperda* to oviposit and live on "Lloyd George" variety of raspberry, which is one of the three varieties known to be attacked by *T. theobaldi*: the others are Bath's Perfection and Reader's Perfection.

In 1938, trials were made with the hawthorn stem midge using three varieties of rose—Southport, Lady Pirie and Inchiquin—three pots of "Lloyd George" variety of raspberry, and four pots of common hawthorn. In every case slits in the stems were made with a scalpel.

In two pots of hawthorn only one female midge was used in each, in the third 3 females and in the fourth 10 females. Egg-laying was seen in each pot of hawthorn several times. The female midges were observed walking up and down the stems till they found a slit and then oviposition took place in the slits. Later examination of the slits revealed larvae in each pot. Adults emerged successfully in two pots, in the third a layer of sand was placed over the soil and no emergence took place, and in the fourth there was very serious overcrowding and the bush died while the larvae were still small.

In both the pots containing Southport rose trees 4 female midges were used, in the Lady Pirie pot 1 female was placed and in the Inchiquin pot 3 females. Although the pots were under continual observation until dark, no oviposition was seen, though one female, which was empty of eggs, was taken from one of the Southport pots after it had been in the cage for 48 hr. No larvae were found in any of the slits and no adult midges subsequently developed.

In one of the raspberry pots 1 female was used and, in the other two, 3 female midges. Here again no oviposition was observed and the females all appeared full of eggs until they died. No larvae were found in the slits in the stems and no adult midges subsequently developed.

Conclusions

The three midges, *T. oculiperda* Rübsaamen, *T. theobaldi* Barnes, and the hawthorn stem midge, form a group of midges which are morphologically very difficult, if not impossible, to separate when one is dealing with numerous specimens. Biologically they have many common attributes but there are differences, e.g. the number of generations and

host plants. Attempts have been made without success to extend the host plant range of two of the three species under consideration.

The evidence, therefore, indicates that the three midges should be regarded as distinct species and this necessitates describing the hawthorn stem midge as a new species.

The name *Thomasiniana crataegi* sp.n. is proposed for this midge and a description follows.

Description of Thomasiniana crataegi *sp.n.*

Male. Length 1½–2½ mm. Antennae: 2+12, first and second flagellar segments fused, each flagellar segment consisting of basal subglobular node bearing one ring of circumfila with regular loops reaching nearly to the base of distal node and a whorl of stout long setae twice as long as loops, and a distal elongated node bearing two rings of circumfila, in the basal ring the loops extend as far as the points of attachment of the distal ring of circumfila, those of distal ring longer, reaching the basal node of the next segment, in addition a whorl of long stout setae distally, slightly longer than the loops of the distal ring of circumfila, these loops being the longest of the three rings; the basal and distal nodes separated by a stem and each distal node bears a distinct neck; the stem of the 3rd flagellar segment is about three-fifths as broad as long and the neck is about twice as long as broad, the neck slightly longer than the stem; stem of 10th flagellar segment about 3 times as long as broad, neck about 4-5 times as long as broad; distal node of 12th flagellar segment bears a distinct and nearly globular elongation. Palpi: proximal segment slightly longer than wide, second about 2½ 3 times as long as wide, third usually about the same, sometimes slightly longer and narrower, distal segment usually distinctly longer and slightly narrower, about 5 times as long as wide. Wings: rather dark owing to being covered with many fine hairs, 3rd vein reaching margin of wing at the tip. 5th vein forked, the two branches forming almost a right angle, the lower branch short. Legs pale with dark hairs, claws bent at right angles, with basal tooth, empodium small. Genitalia: basal clasp segment stout, twice as long as basal width, narrowing distally; distal clasp segment tapering; dorsal lamella with deep narrow V-shaped emargination, each lobe broadly rounded; lower lamella slightly longer than dorsal lamella, with rather shallow U-shaped emargination, each lobe narrowly rounded.

Cotypes: Cecid. 3715–19, 3723–5 and 3748–54.

Other specimen: Cecid. 3223.

Female. Length 2–3 mm. Antennae: 2+12, first and second flagellar segments fused, each flagellar segment cylindrical with short transverse neck, with two almost regular whorls of long stout setae, the setae in the basal whorl much longer than those in the distal, two rings of applied circumfila united by a longitudinal thread, length of 3rd flagellar segment about 2½ times as long as wide, length of 12th flagellar segment about 3 times as long as wide including the distal prolongation which bears long setae. Palps about as in male. Abdomen salmon pink-red, ovipositor long, very extensile, lamelliform, the paired lamellae elongate ovoid, with long fine setae, the basal lamella very small. Otherwise about as in male.

Cotypes: Cecid. 3732–43 and 3745–7.

Other specimen: Cecid. 3224.

Larvae. Salmon pink in colour, breast bone bifid, rounded lobes, shallow rounded emargination, 3–4 mm. long when full grown.

Cecid. 3163–4 and 3185.

Habitat. Under bark of hawthorn twigs.

Locality. Staffordshire, Shropshire, Warwickshire and Hertfordshire.

VI. Summary and conclusions

Emphasis is laid on the need for using biological in addition to morphological characters, in the separation of closely allied species of gall midges.

An analysis is given of the recorded host plant range of species of the genus *Asphondylia*, showing that the great majority of species, 103 out of 133, are recorded from only one species of plant.

Four studies concerning gall midges of economic importance are presented in support of the plea for more intensive biological studies.

The first deals with three supposed species which make terminal leaf galls on various species of *Arabis*. As a result of the study it is concluded that only one true species causes this damage. The species is *Dasyneura alpestris* (Kieffer) de Meijere, while *D. schneideri* Rübsaamen and *D. arabis* Barnes are synonyms. The midge involved attacks certain species of the subgenus *Euarabis* and one other species which may belong to the subgenus *Lomaspora*.

The second study deals with the chrysanthemum gall midge, *Diarthronomyia* sp. Its distribution on commercial chrysanthemums is traced from its initial record as a pest in the United States of America and Canada in 1915. It appeared in England in 1927 and again in 1936, on both occasions the infestation being traceable to chrysanthemum varieties imported from the U.S.A. More recently it has appeared in Denmark (1934), in Sweden (1935), in Finland (1936) and in Northern Ireland (1937). Experiments on the host plant range are described. Oviposition, only, took place on nearly all the *Chrysanthemum* spp. subjected to trial. Galls were formed on *C. Parthenium*, but no adults developed. The midge was bred successfully only on commercial varieties of autumn chrysanthemums, *C. indicum* L., *C. rubellum* Sealy, *C. indicum* var. *azaleoides* (*C. azaleanum* Hort.) and *C.* Korean Apollo. In addition, eggs were laid on such plants as *Matricaria inodora*, *Anthemis nobilis*, *A. Cotula*, *Achillea Millefolium* and *Senecio vulgaris*: in these cases no galls were formed. The conclusion is reached that the midge of commercial chrysanthemums should be regarded as distinct and separate from *Diarthronomyia hypogaea* F.Lw. of wild *Chrysanthemum* spp. recorded from Central Europe.

The third study involves three species of the genus *Dasyneura*, whose larvae cause the leaves of *Rubus* spp., *Ribes nigrum* and *R. Grossularia* to become twisted and crinkled. Attempts to induce *Dasyneura tetensi* Rübsaamen of black currant to attack gooseberry always failed. *D. tetensi* would not mate with *D. plicatrix* H.Lw. of blackberry. In spite of two records in the literature of *D. tetensi* attacking gooseberry, the conclusion is reached that the three species are genuine species—*D. tetensi* Rübsaamen confining its attention to black currant, *D. ribicola* Kieffer limiting itself to gooseberry, and *D. plicatrix* H.Lw. only attacking species of *Rubus*.

The fourth study describes the biology of the newly discovered hawthorn stem midge and compares its bionomics with those of the red bud borer (*Thomasiniana oculiperda* Rübsaamen) and the raspberry cane midge (*T. theobaldi* Barnes). It is concluded that the three midges are distinct species and a description is added of the hawthorn stem midge, to which the name *Thomasiniana crataegi* sp.n. is given.

In addition to those persons whose assistance has already been acknowledged in the course of this paper, the writer wishes to take this opportunity of expressing his gratitude to Miss F. L. Stephens, British Museum (Nat. Hist.), who examined critically the manuscript and made some valuable suggestions which have been adopted. Finally, various discussions with colleagues on the Staff of the Entomology Department at Rothamsted have been of much value.

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Fig. 1.

Fig. 2.

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EXPLANATION OF PLATE XXII

Fig. 1. Galls of chrysanthemum midge on *Chrysanthemum indicum* L.

Fig. 2. Galls of chrysanthemum midge on *Chrysanthemum rubellum* Sealy (*erubescens* Hort.).

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AN APPARATUS FOR TESTING AND COMPARING THE BIOLOGICAL ACTION OF INSECTICIDES ON FLYING INSECTS AND A METHOD FOR SAMPLING THE CONCENTRATION OF THE ATOMIZED INSECTICIDE

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(With 8 Text-figures)

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Introduction

A considerable number of appliances and methods have been described for testing fly sprays. Such an apparatus or method should reproduce as nearly as possible the conditions likely to occur in practice, and of the published methods only those of Peet-Grady (1928) and its modifications and Richardson (1931) appear to fulfil this condition.

The Peet-Grady method has been adopted as a standard in America (see *Soap Blue Book*, 1938, pp. 145, 147, 153), but it seemed to us that Richardson's apparatus was better for a number of reasons. It is not so unwieldy, it can be cleaned more easily and a fan is used to maintain an even temperature and to distribute the insecticide. An apparatus was therefore constructed with Richardson's as a model, but with some slight alterations.

This preliminary apparatus was used to test the emulsified fly sprays, and some of the results are given to show the type of toxicity curves that were obtained. When analysed these results indicated that considerable care must be used in their interpretation.

The major fact which emerged from this work was the necessity of evolving some method of sampling the concentration of the insecticide in the free space of the chamber. If this is not done it is impossible to compare insecticides in different carriers, or to form any estimate of the effect of alteration of the degree of atomization which invariably occurs.

Some further changes were made in the apparatus to ensure that the insecticide was evenly distributed and applied, to facilitate cleaning and to eliminate any chance of toxic residues being left in the chamber. A sampling method was then worked out and tested with three insecticide carriers under a number of different conditions.

Preliminary experiments

The following is a brief description of the first apparatus. It consisted of a rectangular box completely lined with lead and glass to facilitate cleaning. A large slow-moving fan was placed at one end to distribute the insecticide and maintain an even temperature. The apparatus was heated by woven wire resistance mats beneath the glass floor. The inner cage which contained the flies under test was of open mesh tinned gauze and measured $75 \times 45 \times 45$ cm.

The atomizer was an Aerograph "E" model air brush placed in the centre of the end opposite the fan. To avoid excessive coating of one end of the cage, this was placed against the end of the apparatus carrying the atomizer, the tip of which was inserted into the cage so that the insecticide was atomized directly into the cage and then circulated throughout the chamber.

This apparatus was used to test the effect of varying the proportion of oil-in-water emulsions of oil and 4% β-butoxy-β'-thiocyanodiethyl-ether. The oil used was White May kerosene of the following specification: sp. gr. 0·785, flash-point (closed) 125° F., initial boiling-point 170° C., final boiling-point 275° C., percentage distilling at 200° C. = 20. The emulsifier was supplied by Universal Emulsifiers, Ltd., and consisted of inert alumina.

The test insects were *Phormia terraenovae* R.-D. which were bred in the laboratory. They were not standardized with reference to sex but were roughly of the same age.

Table I and Fig. 1 show the results of these experiments. Four experiments were done at each concentration and approximately 100 flies were used for each experiment.

It will be seen from the figures that, under the conditions of the experiment, increasing the percentage of oil in the emulsion caused a

Table I. *The rate of paralysis of flies* Phormia terraenovae *R.-D. caused by atomized emulsions of* 4% *β-butoxy-β′-thiocyanodiethylether in water containing varying proportions of White May kerosene. The insecticide was emulsified with inert alumina and* 1·5 *c.c. were atomized into the test chamber.*

Interval after spraying (sec.)	Log of interval	100% oil		60% oil		50% oil		40% oil	
		% paralysed	Probit	% paralysed	Probit	% paralysed	Probit	% paralysed	Probit
60	1·7782	5	3·3551	4	3·2493	2	2·9463	2	2·9463
120	2·0792	39	4·7207	27	4·3872	15	3·9636	12	3·8250
180	2·2553	75	5·6745	62	5·3055	45	4·8743	36	4·6415
240	2·3802	94	6·5548	82	5·9154	79	5·8064	57	5·1764
300	2·4771	97	6·8808	94	6·5548	88	6·1750	67	5·4399
360	2·5563	99	7·3263	96·5	6·8119	89·5	6·2536	74	5·6433
420	2·6232	—	—	—	—	90·5	6·3106	81	5·8779
480	2·6812	—	—	—	—	—	—	85	6·0364
540	2·7324	—	—	—	—	—	—	—	—
600	2·7782	—	—	—	—	—	—	—	—
660	2·8195	—	—	—	—	—	—	—	—
720	2·8573	—	—	—	—	—	—	—	—

Interval after spraying (sec.)	Log of interval	30% oil		20% oil		10% oil	
		% paralysed	Probit	% paralysed	Probit	% paralysed	Probit
60	1·7782	0	—	0	—	0	—
120	2·0792	6	3·4452	4	3·2493	2	2·9463
180	2·2553	23	4·2612	21	4·1936	12	3·8250
240	2·3802	42	4·7981	36·5	4·6549	24	4·2937
300	2·4771	59	5·2275	48	4·9498	39	4·7207
360	2·5563	64	5·3585	59	5·2275	53	5·0753
420	2·6232	70	5·5244	63	5·3319	59·5	5·2404
480	2·6812	72·5	5·5978	71	5·5534	65	5·3853
540	2·7324	75·5	5·6903	76	5·7063	69	5·4959
600	2·7782	77·5	5·7554	80·5	5·8596	72	5·5828
660	2·8195	—	—	83	5·9542	79	5·8064
720	2·8573	—	—	—	—	75	5·6745

marked increase in toxicity over a range of 10–50%, but the increase in toxicity was much more gradual over the range 50–100% oil.

The results were originally plotted as the percentage paralysed against the time of exposure, when sigmoid curves were obtained. However, when the percentage paralysed was converted to probits and plotted against the logarithm of the time of exposure, the points for each concentration of oil did not always lie along a straight line. They seemed, in the lower concentrations, to be best fitted by two straight lines, the break appearing towards the end of the exposure period. The lines shown are only the freehand provisional regression lines.

In these experiments, the concentration of the insecticide in the chamber is continuously falling, so that a straight line relationship

between the exposure and the number paralysed is not to be expected. The explanation of the fact that the points do not lie on a pronounced curve may be that, after the initial rapid fall, the concentration of insecticide falls off gradually so that, for a period, the ordinary straight line dosage-mortality relationship holds, but at some point in the exposure

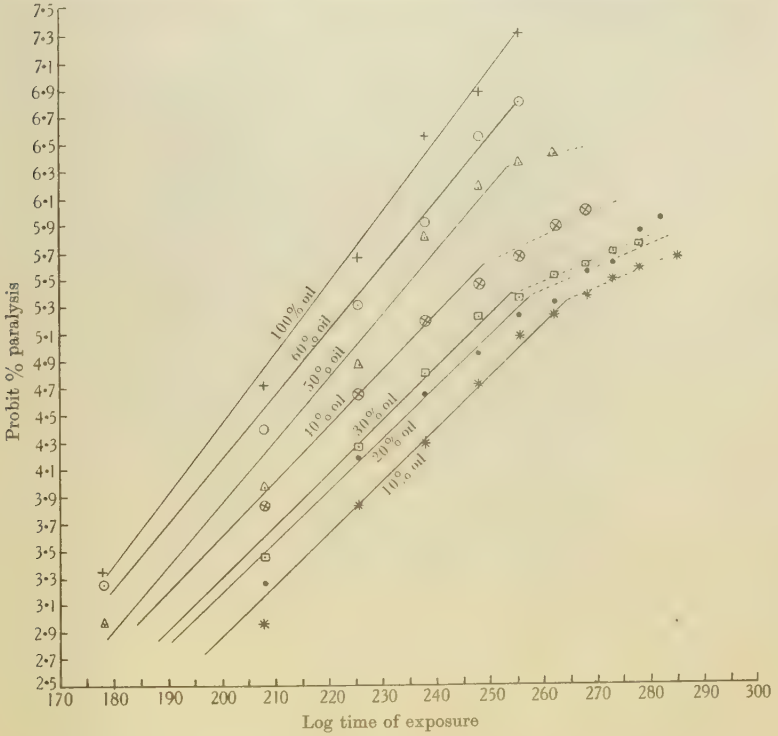


Fig. 1. Graph from Table I showing the effect of emulsions of 4% β-butoxy-β′-thiocyanodiethylether in water containing varying proportions of White May kerosene on *Phormia terraenovae* R.-D. 1·5 c.c. of insecticide atomized into the chamber.

the concentration falls below a threshold value and a break in the line occurs. The reason for no break occurring in the high percentages of oil may be, that they were atomized more finely and the concentration did not fall below the threshold value during the time of exposure allowed.

It became evident during the course of these experiments that insufficient data were available to give a satisfactory interpretation of the results. The lowering of toxicity, with decreasing percentages of oil

in the emulsion, might equally well be due to a more rapid fall in concentration of the insecticide in the chamber (due to less fine atomization) as to an actual decrease in the toxicity of the emulsion.

Further, it appeared that a method of measuring the concentration of the atomized insecticide in the chamber was essential, in order to compare insecticides in carriers of different physical properties and in order to standardize the degree of atomization with any given carrier.

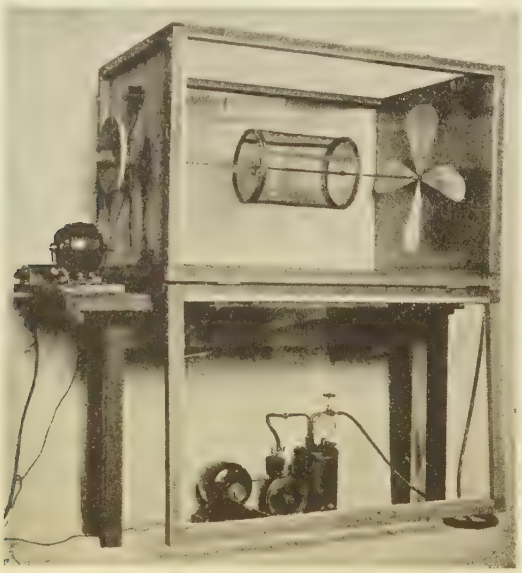


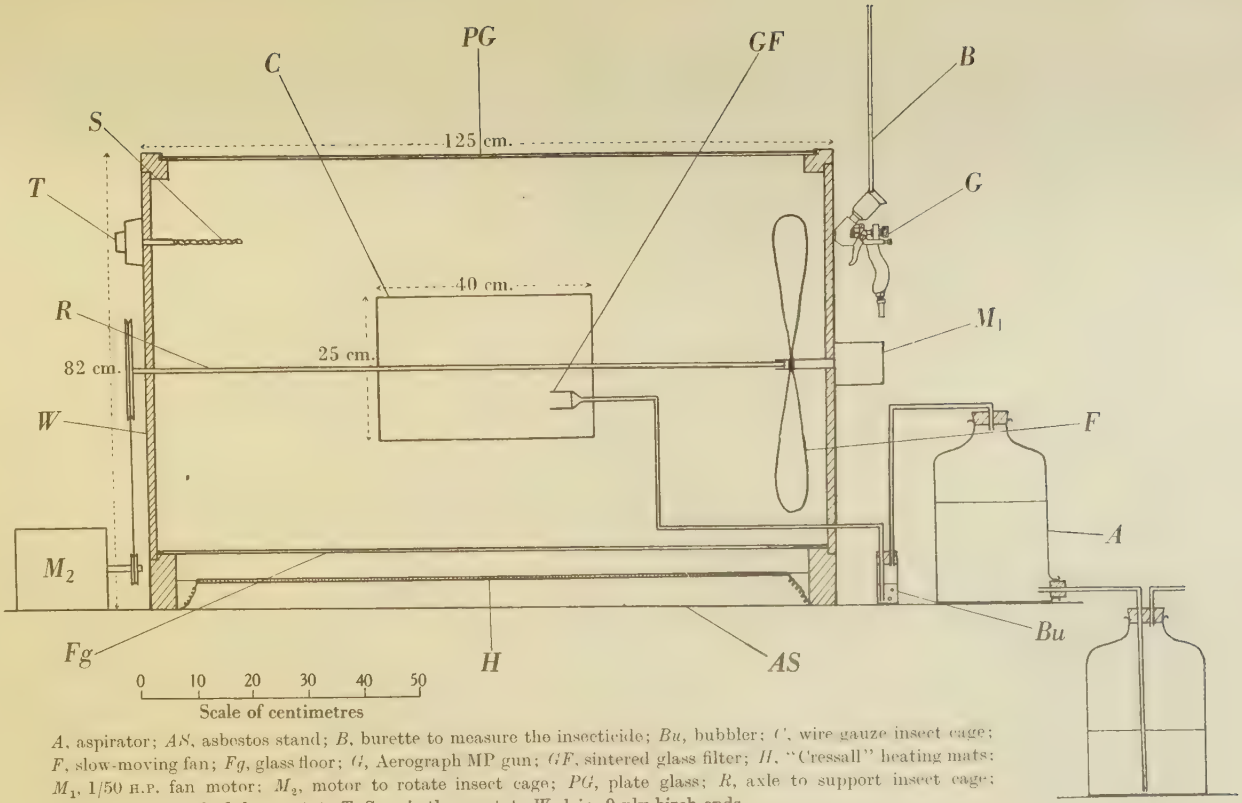
Fig. 2. Photograph of testing apparatus finally adopted.

In addition, some modifications of the apparatus were desirable. The disadvantages of the original apparatus were, first, that the flies had to be introduced before spraying and were liable to get a heavy dose of insecticide before it was properly distributed; secondly, that different parts of the cage containing the flies received very different deposits of spray so that the flies crawling on it were variously affected.

An apparatus was therefore constructed to overcome these two defects and a sampling technique was also worked out.

Description of the final apparatus (Figs. 2, 3)

The testing chamber measured $4 \times 2\frac{1}{2} \times 2\frac{1}{2}$ ft. with a framework of $2 \times 1\frac{1}{2}$ in. deal. The back, top and hinged front were made of 32 oz. plate glass (*PG*) and the ends of $\frac{1}{2}$ in. 9-ply birch (*W*). The floor (*Fg*) was



A, aspirator; *AS*, asbestos stand; *B*, burette to measure the insecticide; *Bu*, bubbler; *C*, wire gauze insect cage; *F*, slow-moving fan; *Fg*, glass floor; *G*, Aerograph MP gun; *GF*, sintered glass filter; *H*, "Cressall" heating mats; M_1, 1/50 H.P. fan motor; M_2, motor to rotate insect cage; *PG*, plate glass; *R*, axle to support insect cage; *S*, bimetallic spiral of thermostat; *T*, Sunvic thermostat; *W*, $\frac{1}{2}$ in. 9-ply birch ends.

Fig. 3. Transverse section of chamber showing sampling apparatus in position.

of 32 oz. plate glass supported by $\frac{1}{2}$ in. birch plywood, in which five parallel rectangular openings 3×4 in. were cut to expose five woven wire "Cressall" heating mats (*H*), each of 42 ohms resistance and wired to take 1 kW. The chamber rested on a sheet of 3/16 in. asbestos (*AS*). The temperature was controlled (within $\pm 1°$ C.) by a type TS Sunvic thermostat (*T*) fixed with a relay on the outside of the left wall with the bimetallic spiral (*S*) projecting into the chamber. All internal wood surfaces were covered with heavy (7 oz./sq. ft.) lead foil.

A 2 ft. diameter fan (*F*) was fixed centrally on the right-hand wall and was revolved in a counter clockwise direction at approximately 100 rev./min. by a 1/50 H.P. electric motor (M_1) which was outside the chamber.

The insecticide was sprayed in at a point 5 in. from the top and front on the right-hand end by a type MP Aerograph spray gun (*G*) with a no. 1 nozzle worked by a small air compressor shown in Fig. 2.

A cylindrical open mesh (8 to the inch) brass gauze cage (*C*), 10 in. in diameter and 15 in. long, was suspended in the centre of the chamber on a 3/8 in. axle (*R*) which pivoted in the centre of the fan and passed through the left-hand end. The cage could be slowly rotated in the reverse direction to the fan by a fractional H.P. electric motor (M_2) outside the chamber. A cage of smaller mesh is necessary if mosquitoes are used.

Spraying technique

With the chamber closed and the fan and gauze cage (*C*) rotating in opposite directions, a known quantity of insecticide (usually 3 c.c.) was sprayed in from burette *B* under a known air pressure (usually 30 lb./sq. in. recorded on the gauge of the air compressor) in a known time. The time was checked by a stop-watch. After 4 min., when the mist was distributed and the initial rapid fall in concentration had taken place, the rotation of the cage was stopped and a glass tube 2 ft. long and 1 in. in diameter, containing fifty flies, was put horizontally through a 1 in. hole in the left end of the chamber and through a hole in the left end of the cage. The flies were gently pushed out of the tube and into the cage with a perforated piston. The tube and piston were then withdrawn and the hole closed with a bung (see below for method of introducing mosquitoes).

The number of flies knocked down and paralysed at $\frac{1}{2}$ min. intervals was counted.

When the flies had been in the mist for 15 min. the chamber was

opened, the cage removed, one end taken off and the flies gently shaken into a jar where they were left for 24 hr. with sugar solution as food and drink. The numbers completely dead and moribund at the end of that time were recorded.

The whole of the chamber and the cage was thoroughly wiped with acetone, and after airing for about half an hour the apparatus was ready for further use.

Control experiments indicated that this was a satisfactory method of cleaning the apparatus, but it would be preferable to have at least two cages, so that one could be thoroughly cleaned while the other was in use.

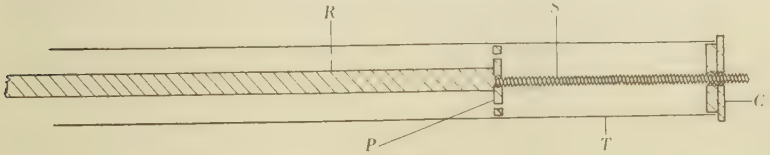


Fig. 4. Apparatus for introducing mosquitoes into the testing chamber. *C*, screw-on cap; *P*, perforated piston; *R*, push rod; *S*, mosquito chamber; *T*, glass tube.

Mosquitoes were found to be too fragile to be pushed along the glass tube so the arrangement shown in Fig. 4 was used. Fifty mosquitoes were put in the space (*S*) and held in by the perforated piston (*P*) and the screwed on cap (*C*). The glass tube (*T*) was inserted through the 1 in. holes into the cage inside the chamber, a slight movement of the rod (*R*) pushed the cap (*C*) away from the tube, and the mosquitoes escaped into the cage.

This apparatus was used for tests on flies and mosquitoes but no attempt was made to obtain a sequence of results and the figures are not given here. Once the apparatus had been completed and found satisfactory, attention was given to working out a method of sampling, and then to using it to obtain data on the behaviour of the insecticide after it had been atomized into the chamber.

Technique of sampling concentrations of insecticide in the test chamber

The first method tried was to suck a measured quantity of the atmosphere in the chamber through a substance chosen to absorb the insecticide carrier. The substance was weighed before and after absorption to obtain the weight of the carrier in the given volume of air. This method usually gave low results, which were probably largely due to the

volatilization of the carrier, the vapour of which was not absorbed, and also to changes in the water vapour content of the absorbent material.

A second method was tried which proved successful. This was to dissolve a dye in the carrier which was then atomized into the chamber; the concentration was sampled when desired by sucking a measured quantity of the atmosphere of the chamber through a sintered glass filter which retained the particles of dye. The retained dye was then washed out of the filter with a measured quantity of undyed carrier and the colour matched with a set of standards in order to ascertain the amount of dye that had been absorbed.

The method is thought to give a good index of the concentration of the insecticide and would also serve to measure the distribution. It is independent of the vaporization of the carrier. With any given carrier it can be used to determine the rate at which the concentration of insecticide falls off and so the relative degree of atomization. The method assumes that the particles of insecticide will behave in a similar manner to the dye particles, and several dyes were tried before the final choice was made. All the dyes behaved in a similar manner, but those chosen were the best for colour comparison. Because the different dye molecules behaved in a similar way, it is reasonable to assume that the insecticide molecules would also behave similarly in this respect.

In addition to being a measure of the concentration of the insecticide itself, the method indicates the behaviour of the carrier after atomization. The percentage of dye remaining suspended is also the percentage of carrier that has not settled out, but remains in the atmosphere either in its original atomized state or as a vapour.

The most satisfactory dyes used were Sudan III for oils, and methylene blue for water. Colour standards were made up in ten glass tubes ($2 \times \frac{3}{8}$ in.). The tubes contained from 0·001 to 0·01 c.c. of the coloured base with sufficient uncoloured oil or water added to make each one up to 3 c.c. The arrangement of the sampling apparatus is shown in Fig. 3.

A Jena sintered glass filter funnel (Büchner type) (*GF*) of 30 mm. diameter and with pores of 15–40μ was placed inside the cage and connected by glass tubing ($\frac{1}{4}$ in. diameter) to an aspirator (*A*) outside the chamber.

3 c.c. of the dyed liquid were sprayed in under 30 lb./sq. in. air pressure in a known time and with the fan rotating. After a definite recorded interval (from 10 sec. to 30 min.) the aspirator tap was opened and 2 l. of the atmosphere of the cage were drawn through the sintered

glass filter, which held all the dye particles. The filter had been tested to ascertain whether the dyes passed through it, by putting a cotton-wool pad beyond it and examining it afterwards for traces of colour.

The dye was washed out with 3 c.c. of spray base into a 2 by $\frac{3}{8}$ in. glass tube and the resulting colour compared with the standards.

It was found expedient with the water base experiments using methylene blue as a dye to dilute the standard and wash out the filter with alcohol, as water did not dissolve the dye rapidly enough.

The capacity of the whole chamber was 600 l. and 1/300th of this was removed. That this method of measurement does not result in any significant dilution of the sample by air entering the chamber to replace that which is drawn out is shown by using the equation

$$x = \frac{(ab\,(m-1) - (a-b)\,e^{-m})}{mv},$$

given by Page & Lubatti (1937) in estimating dilution effects on the sampling of fumigants. In this instance v = the volume of the chamber = 600 l.; mv = the volume of the sample = 2 l.; $m = mv/v = 0{\cdot}003$, a/v = the initial concentration of the atomized material, b/v = the concentration of the atomized material in the outside space, x = the determined concentration of the atomized material in the sample. Since $b = 0$ the equation may be written

$$x = \frac{a\,(1-e^{-m})}{mv},$$

$$\frac{x}{a/v} = \frac{(1-e^{-m})}{m} = \frac{0{\cdot}0032945}{0{\cdot}0033333},$$

that is, under the given conditions, the ratio between the actual concentration and the concentration determined for all concentrations of atomized spray inside the chamber = 1/1·01, which, expressed as a percentage, = 98·8%. Since the colorimetric readings were only accurate to within 10% it is not necessary to correct for this sampling error due to dilution.

The colour standards contained 0·001, 0·002, 0·003, etc., up to 0·010 c.c. of the coloured spray and the resulting colours given by the aspirated sample therefore corresponded with these standards when the whole chamber contained 300 times this amount of spray, i.e. 0·3, 0·6, 0·9, etc., up to 3 c.c. Therefore standards 1–10 corresponded to 10, 20, 30, etc., up to 100% of the 3 c.c. originally sprayed in.

For more accurate measurements a colorimeter would be necessary,

but it was found possible to judge within 10% with certainty with the method described.

Some experiments were done using White May kerosene, the specification of which has already been given: and some with Shell oil 24210, a white oil of the following specification: sp. gr. = 0·862, flash-point (closed) 320° F., flash-point (open) 335° F., visc. redw. 1 at 70° F. = 118 sec., pour test = −30° F., unsulphonated residue = 99·2% by volume and sufficiently refined to be odourless and nearly tasteless; and water.

Table II. *The rate of settling out of a light oil (White May kerosene) stained with Sudan III. Each of series 1–6 shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray 3 c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of oil atomized in 4½ sec.			Series 2: 3 c.c. of oil atomized in 6½ sec.		
6 sec.	1·65	55	10 sec.	2·1	70
30 sec.	1·13	38	30 sec.	1·75	58
60 sec.	1·05	35	—	—	—
2 min.	0·92	31	2 min.	1·10	37
5 min.	0·62	21	5 min.	0·87	29
10 min.	0·45	15	10 min.	0·76	25
20 min.	0·35	11	20 min.	0·54	18
Series 3: 3 c.c. of oil atomized in 10 sec.			Series 4: 3 c.c. of oil atomized in 13½ sec.		
7 sec.	2·5	83	9 sec.	2·76	92
30 sec.	2·4	80	30 sec.	2·73	91
60 sec.	2·2	73	60 sec.	2·60	87
2 min.	1·9	63	2 min.	2·30	77
5 min.	1·6	53	5 min.	1·70	57
10 min.	1·2	40	10 min.	1·30	43
20 min.	0·95	32	20 min.	1·05	35
Series 5: 3 c.c. of oil atomized in 20 sec.			Series 6: 3 c.c. of oil atomized in 65 sec.		
9 sec.	2·76	92	7 sec.	2·79	93
—	—	—	—	—	—
60 sec.	2·72	91	60 sec.	2·73	91
2 min.	2·67	89	2 min.	2·70	90
—	—	—	5 min.	2·55	85
10 min.	2·16	72	10 min.	2·40	80
20 min.	1·73	58	20 min.	2·10	70

Table II and Fig. 5 show how dye particles carried in White May kerosene settle out at different degrees of atomization. The degree of atomization was adjusted by keeping the air pressure constant, but adjusting the liquid needle valve so that the 3 c.c. went out at different speeds. The faster the liquid went out the coarser the degree of atomization. It is evident that the degree of atomization has a very marked effect on the amount of material remaining suspended in the atmosphere. With the comparatively coarse atomization of series 1 and 2 there is an initial

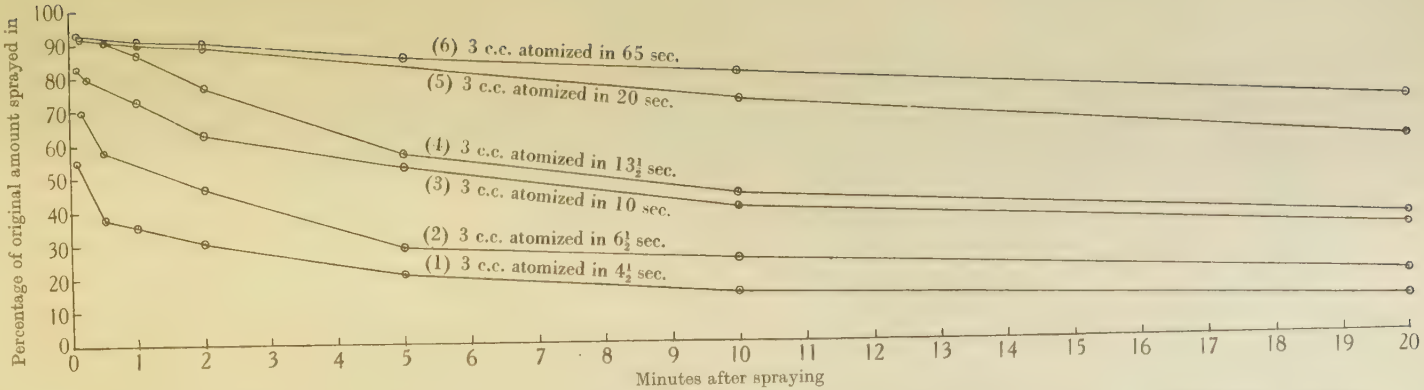


Fig. 5. Graph showing the rate of settling out of the colour when a light oil (White May kerosene) stained with Sudan III is atomized into a chamber. Each of series 1–6 shows the rate of settling out at a different degree of atomization. See Table II.

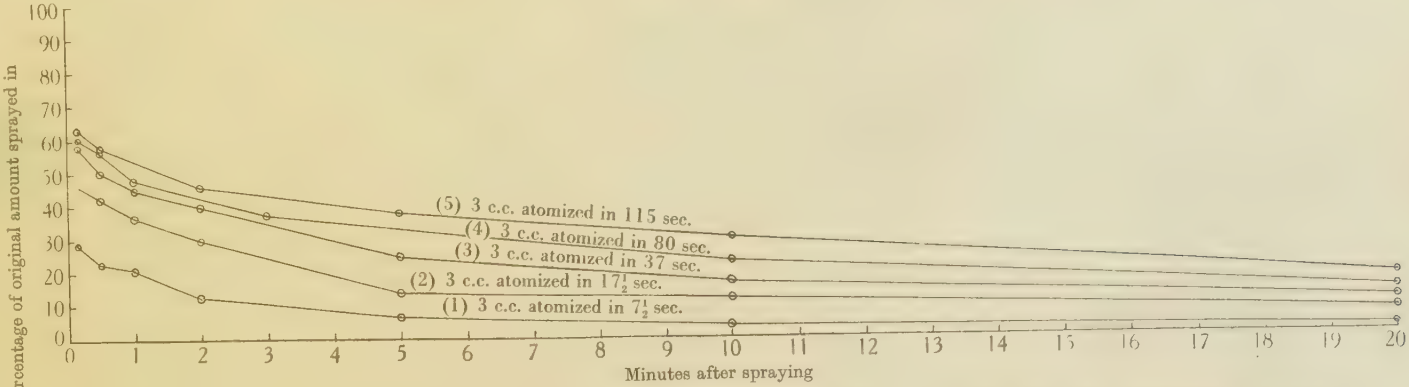


Fig. 6. Graph showing the rate of settling out of the colour when a white oil (Shell oil 24210) stained with Sudan III is atomized into a chamber. Each of series 1–5 shows the rate of settling out at a different degree of atomization. See Table III.

rapid fall in concentration, probably produced by the larger droplets settling out, followed by a steady slower drop. In series 3–6 this initial drop is not so marked and a high proportion of the material is still suspended at the end of 2 min. The proportion at the end of 20 min. increases with increasing fineness of atomization. From the point of view of the testing of insecticides these figures show that consistent and comparable results cannot be expected unless the degree of atomization is standardized either by sampling or some other method. They also show that unless the insecticide is finely atomized, the time at which the insects are introduced is important, because during the first 30 sec. the concentration is falling rapidly and unless the insects are present throughout the spraying, or are introduced at exactly the same time on every occasion, or are introduced after the first 30 sec., the concentrations to which they are exposed will be very different.

Table III. *The rate of settling out of the white oil (Shell oil 24210) stained with Sudan III. Each of series 1–5 shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray 3 c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of oil atomized in 7½ sec.			Series 2: 3 c.c. of oil atomized in 17½ sec.		
10 sec.	0·87	29	10 sec.	1·37	46
30 sec.	0·70	23	30 sec.	1·26	42
60 sec.	0·62	21	60 sec.	1·10	37
2 min.	0·40	13	2 min.	0·90	30
5 min.	0·22	7	5 min.	0·43	14
10 min.	0·11	4	10 min.	0·35	12
20 min.	0·06	2	20 min.	0·22	7
Series 3: 3 c.c. of oil atomized in 37 sec.			Series 4: 3 c.c. of oil atomized in 80 sec.		
10 sec.	1·75	58	10 sec.	1·80	60
30 sec.	1·50	50	30 sec.	1·70	57
60 sec.	1·35	45	60 sec.	1·45	48
2 min.	1·20	40	2 min.	—	—
5 min.	0·76	25	3 min.	1·10	37
10 min.	0·50	17	10 min.	0·70	23
20 min.	0·30	10	20 min.	0·40	13
Series 5: 3 c.c. of oil atomized in 115 sec.					
10 sec.	1·88	63			
30 sec.	1·73	58			
60 sec.	—	—			
2 min.	1·37	46			
5 min.	1·15	38			
10 min.	0·90	30			
20 min.	0·50	17			

Table III and Fig. 6 show the results of an experiment with Shell oil 24210 similar to the above. The same general results are illustrated in this experiment as in the preceding one.

The most important additional point that this experiment demonstrates is the difficulty of comparing insecticides in different carriers. A dye in White May kerosene sprayed in 6½ sec. remains suspended better than the same dye in Shell oil 24210 sprayed in 115 sec. under the same conditions. From these results it is clear that where insecticide carriers of different physical properties are being used, it is not possible to make a fair comparison of the toxicity unless some method such as sampling is adopted to ensure that equivalent amounts of the toxic material remain in suspension.

This experiment also shows that the heavy oil is more difficult to atomize satisfactorily. In order to break up the particles sufficiently finely within a reasonable space of time it would be necessary to use a higher air pressure than that used in these experiments.

Table IV. *The rate of settling out of water stained with methylene blue in an unsaturated atmosphere. Each of series* 1–4 *shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray* 3 *c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of water atomized in 5 sec.			Series 2: 3 c.c. of water atomized in 10 sec.		
10 sec.	1·2	40	10 sec.	2·05	68
20 sec.	1·05	35	60 sec.	1·95	65
2 min.	1·0	33	2 min.	1·86	62
5 min.	0·95	31·5	5 min.	1·70	56·5
10 min.	0·84	28	10 min.	1·75	58
20 min.	0·80	26·5	20 min.	1·70	56·5
Series 3: 3 c.c. of water atomized in 23 sec.			Series 4: 3 c.c. of water atomized in 57 sec.		
10 sec.	2·5	83	10 sec.	2·92	97
60 sec.	2·0	66·5	60 sec.	2·86	95
2 min.	1·95	65	2 min.	2·60	86·5
5 min.	—	—	5 min.	2·40	80
10 min.	1·95	65	10 min.	2·0	66·5
20 min.	1·75	58	20 min.	1·97	65·5

The results of the experiments using a water base stained with methylene blue are presented in Tables IV and V and Figs. 7 and 8. Both these sets of results confirm the general conclusions of the previous experiments. They also show that when a water base is used the amount remaining suspended in the atmosphere under given conditions of atomization depends on the initial degree of saturation of the atmosphere as well as on the degree of atomization. The material settles out far more rapidly in a saturated atmosphere than in an unsaturated one.

The primary object of the chamber method of testing and comparing the toxicity of insecticides to flying insects is to expose the insects to a

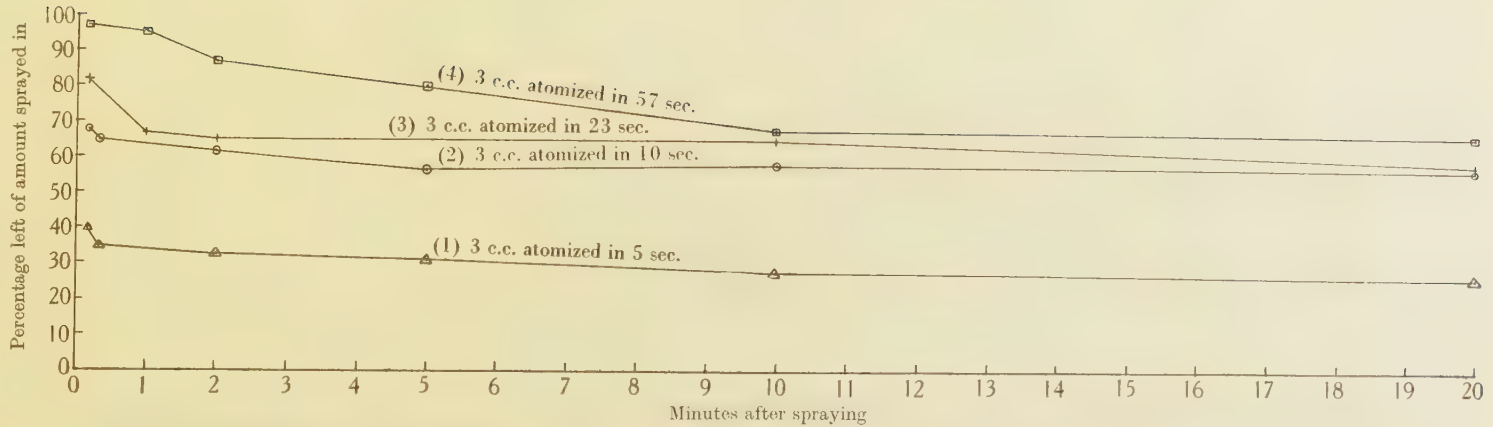


Fig. 7. Graph showing the rate of settling out of the colour when water stained with methylene blue is atomized into an unsaturated atmosphere. Each of series 1–4 shows the rate of settling out at different degrees of atomization. See Table IV.

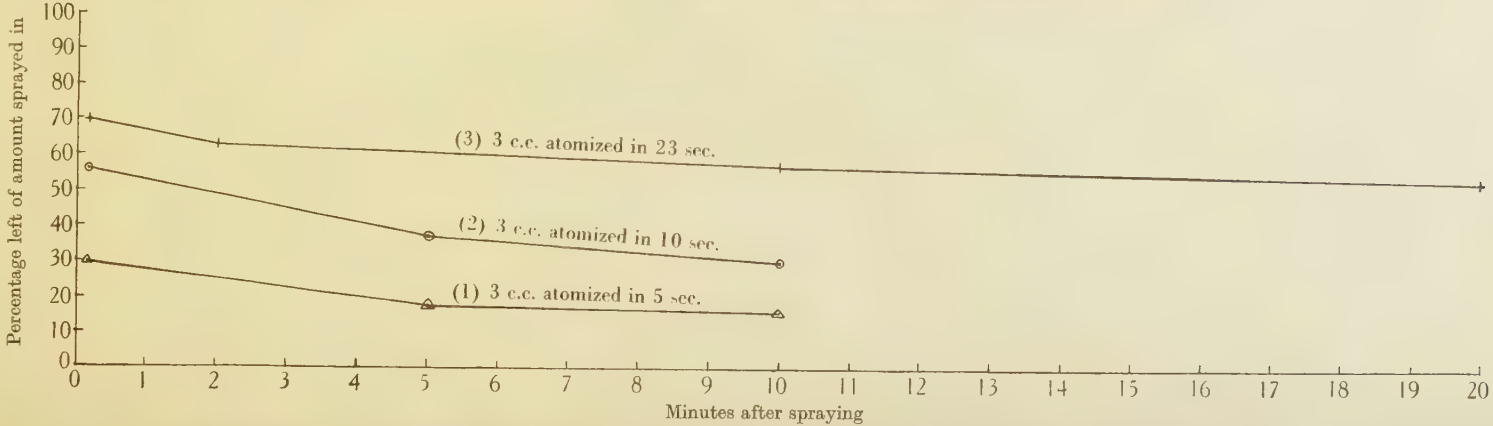


Fig. 8. Graph showing the rate of settling out of the colour when water stained with methylene blue is atomized into a saturated atmosphere. Each of series 1–3 shows the rate of settling out at different degrees of atomization. See Table V.

Table V. *The rate of settling out of water stained with methylene blue in a saturated atmosphere. Each of series* 1–3 *shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray* 3 *c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of water atomized in 5 sec.			Series 2: 3 c.c. of water atomized in 10 sec.		
10 sec.	0·90	30	10 sec.	1·70	56·5
5 min.	0·54	18	5 min.	1·10	36·5
10 min.	0·48	16	10 min.	0·90	30
Series 3: 3 c.c. of water atomized in 23 sec.					
10 sec.	2·10	70			
2 min.	1·90	63			
10 min.	1·72	57			
20 min.	1·60	53			

given quantity of the material suspended in the atmosphere of the chamber under standard conditions. These experiments show that it is difficult if not impossible to do this continuously unless a sampling method is adopted. If the same carrier is utilized throughout, a slight change in the atomizer may alter considerably the amount remaining suspended after a given time. Unless the insects are kept in the chamber throughout the spraying, which has the disadvantage that they are exposed to an unequally distributed insecticide, it is desirable to know when the initial rapid fall in the insecticide has taken place in order that the insects may be introduced after it has occurred. If different carriers are being used it is essential when making comparisons that the behaviour of each carrier after it has been atomized should be known.

The sampling methods described above should make it possible to obtain considerably more information than is at present available on the behaviour of atomized insecticides, always providing that the dye molecules behave in a similar manner to the insecticide molecules.

Summary

1. An apparatus and method for testing the effect of atomized sprays on flies and mosquitoes are described. The apparatus consists of a revolving wire gauze cage placed in a thermostatically controlled chamber, the whole of which may be easily cleaned and freed from toxic residues. The insecticide is sprayed into the chamber by means of an Aerograph MP gun and distributed by means of a slow-moving fan. When the insecticide has been injected and an interval allowed for the initial rapid fall of concentration, the movement of the cage is stopped and the

insects are introduced into it by means of a special tube and plunger. The time required for paralysis to take place is recorded. After a given interval the insects are removed from the gauze cage and kept to ascertain the mortality.

2. A technique for sampling the concentration of insecticide in the air space is described. The insecticide carrier is coloured with a dye, Sudan III for petroleum oil bases and methylene blue for water bases. The percentage of atomized material remaining in the atmosphere at any given time is determined by aspirating a known quantity of the atmosphere of the chamber through a sintered glass filter. The dye is retained in the filter, it is washed out with a measured quantity of liquid and compared with known standards. Reasons are given for the assumption that the dye molecules will behave in the same way as the insecticide molecules.

This sampling method has been used to study the behaviour of a light oil, a heavy white oil and water at different degrees of atomization. Tables and graphs are given which show that, except with a fine atomization where most of the insecticide remains suspended for a considerable time, there is an initial rapid fall, which varies in amount with the degree of atomization. This initial fall is followed by a much more gradual decrease of concentration. The experiments show clearly that oil bases of different physical properties cannot be compared adequately unless a sampling method is used to ascertain the quantities of material remaining suspended. Where water bases are used it is shown that the time concentration curve varies not only with the degree of atomization, but also with the degree of saturation of the atmosphere before spraying. The concentration remains higher in an unsaturated atmosphere than in a saturated one.

We are indebted to Prof. J. W. Munro, in whose department the work was carried out, and to Dr A. B. P. Page of this department for many helpful suggestions on the method of sampling.

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BIOLOGICAL METHODS OF TESTING INSECTICIDES

A REVIEW

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Introduction

Insecticides can be divided into three classes: (*a*) contact insecticides, (*b*) stomach poisons, (*c*) fumigants. Those in class (*a*) act by penetration of the integument and tracheal system; in (*b*) by ingestion in the intestinal tract; (*c*) probably react in approximately the same way as the contact insecticides, but through the vapour phase and by a readier access to the tracheal system. The three classes require different methods for their biological evaluation both in the laboratory and the field.

LABORATORY METHODS

(*a*) *Contact insecticides*

These can be divided into the following classes according as the technique involves (i) spraying, (ii) dipping, (iii) the use of the micropipette, (iv) dusting.

Spraying technique.

The methods, involving the use of finely atomized spray fluids, are of two kinds: those in which the liquid is thrown in the form of fine droplets on to the insects on a surface or flying about in a chamber. The latter is usually employed only in work upon household sprays, and houseflies are almost universally employed as test subjects.

Insects on a surface. Tattersfield & Morris (1924) elaborated an apparatus, later modified with respect to its atomizer (Tattersfield, 1934), so arranged that successive batches of insects on a surface could be sprayed under controlled conditions with respect to pressure of spray and quantity of insecticide atomized upon the insects. It consisted of a glass jar with external and internal levelling platforms; upon the latter a small glass dish containing the insects could be placed at a fixed distance from the atomizer, held in the lid by a clamp. The internal platform stood on levelling screws on a glass disk reposing on a small glass tripod and the atomizer was so arranged that the cone of spray could be adjusted to fall as evenly as possible about the dish. The dish of insects was placed in the instrument by removing the lid, and the fluid, already pipetted into a small reservoir, was immediately atomized by means of an air-line connected through a pressure gauge to a cylinder of compressed air fitted with an adjusting valve. Later models were of square section and the insects were introduced through a sliding door in one of the sides. This form of apparatus has been used by Steer (1938). Small sucking insects, e.g. aphides (Tattersfield & Morris, 1924), or insect eggs (Gimingham *et al.* 1926) can be used as test subjects. One of the main advantages of the apparatus consists in the small amount of spray fluid required; it can, however, be criticized on the grounds that the area, over which the deposit of spray is relatively evenly distributed, is small.

Potter (1938, private communication) has constructed a spray apparatus which, although of a similar type to that of Tattersfield & Morris, achieves a greatly improved distribution of the spray fluid over a larger area. It involves the use of a larger amount of spray fluid, and the employment of a mixing tower above the spray chamber. The cone of spray is by this means broken up, and the droplets fall in an evenly dispersed shower over the area upon which the insects are placed.

O'Kane *et al.* (1930), in an important paper on the factors involved in the performance of contact insecticides, described a slightly different type of apparatus, in which the insecticide was sprayed diagonally upon the insects placed upon a rotating disk.

Jones *et al.* (1935) modified these methods with the object of saving time and labour and to obtain greater uniformity of application. The apparatus evolved is now widely known as the Campbell turn-table. Glass cylinders on a turn-table can be moved successively under a spray-gun, the insecticide is sprayed in, the cylinder allowed to stand for a short but definite length of time and then moved laterally over house-flies, just recovering from quiescence induced by chilling or anaesthesia, in a dish covered by wire mesh. In all these methods the effect of the insecticide is judged after certain periods of time, the numbers of insects affected are placed in categories, and the toxic effect produced correlated with the concentration.

Since Campbell's method depends on the deposition on the insect of a falling mist of insecticide, the larger droplets having been got rid of by the initial standing, certain very definite precautions are requisite if results are to be replicated. As first described, the jar with the enclosed mist was moved by hand over the insects; in order that the weight of deposit should be the same in repeat experiments, the time taken and the procedure adopted had to be reproduced with considerable care. Moreover, the use of wire mesh over the insects may give rise to complex eddy currents, and the employment of chilled or anaesthetized flies may introduce factors the effects of which upon insect resistance are imperfectly known. They are perhaps to be deprecated. Zermuehlen & Allen (1936) modified the process by using a removable paper cover over the insect container; this was withdrawn and the container pushed into the jar containing the insecticide mist. Campbell & Sullivan (1938) have recently elaborated an improved turn-table method, and as a result of their investigation have made further suggestions directed towards the improvement, simplification and cheapening of the apparatus. It is constructed entirely of metal, consisting of a triangular frame on which is mounted a circular aluminium turn-table (46 in. diameter). The movable glass cylinders are replaced by aluminium ones (17 in. high and 8 in. diameter), ten in number. Below each of these are cups (4 in. deep and 6 in. diameter) attached to the underside of the table. These hold the cages of insects which can be cut off from the upper cylinder by means of stainless steel slides running in slots in the table. When operating for testing fly-sprays a 5½ in. screen-covered Petri dish containing 100 flies is placed in one of the cage-holders, the slide pushed in to cover it and the larger aluminium cylinders placed over it. A known volume of the appropriate spray fluid to be tested is atomized at constant pressure into the upper cylinder through a hole in the lid, the larger droplets are allowed to settle for a known time, the slide is withdrawn and the fine mist allowed to deposit upon the flies in the cage below. After 10 min. exposure, the Petri dish is removed and the treated flies transferred to observation cages. By turning the table and bringing the spray cylinders successively into action, a number of tests can be carried out with considerable rapidity. The method was primarily designed for testing fly-sprays for which an oil base is used. Badertscher (1936) compared the turn-table method with the standard Peet-Grady technique (see below) and found that he obtained higher percentage kills with the Peet-Grady apparatus for pyrethrum and thiocyanate sprays, a result confirmed by Campbell & Sullivan (1938). Badertscher obtained, however, the curious and apparently anomalous result that the reverse was the case for sprays containing rotenone. Speed in operation and the fact that aqueous sprays can be tested are stressed as advantages of the turn-table method. The method depends upon the settlement of a mist, and as has been pointed out by Campbell & Sullivan (1938) it is possible that, for insects above a certain level of resistance, the maximum deposit of liquid available may be insufficient to secure a satisfactory kill. The authors suggest that this difficulty might be met by spraying directly through the cage containing the insects by removing the disk on the bottom of the cage-holder.

If reliable and replicable results are to be obtained by the use of this apparatus, there is no question that the whole sequence of operations must be carried out in as uniform a way as possible. Not only so, but the insects used should be reared as uniformly as possible.

Other methods depend upon spraying infested plants while they are being slowly

rotated. Hartzell & Wilcoxon (1932) employed such a technique. Immediately after spraying, the plants (nasturtiums) are placed with the pot lying on its side, so that the plant itself lies over squared paper surrounded by a barrier of grease or gummy material. The effect produced was determined by examination under a binocular microscope with probing—any insect showing movement was regarded as alive.

Lethal-chamber methods. These are chiefly used for testing fly-sprays. The Peet-Grady method (1928, 1932) was one of the earliest to be so employed. Essentially the apparatus is a small room, $6 \times 6 \times 6$ ft. inside, constructed of non-absorbent walls with impermeable sealed corners. It is fitted with a tightly flush-fitting entrance door and observation windows in the centre of each wall, the ceiling has a window 18 in. square, 6 in. above which a 200-watt light bulb is set for illumination. In each lower corner a 6×6 in. square port is cut, covered by wire gauze and tight-fitting hatches.[1] Two 1 in. holes are bored in each wall 6 in. from the ceiling, 1 ft. from the edge, which can be closed by corks. A fan and wire-covered hatch for changing the air after each test are fitted opposite the entrance. The technique for operating involves the liberation of flies, of a definite age and reared under as constant a set of conditions as possible, into the chamber and the subsequent spraying, under a definite pressure, of a total of 12 c.c. of the insecticide solution in about equal amounts through the eight 1 in. holes. After 10 min. the ventilating fan is started, after opening the screen ports, and the flies carefully taken out, counted, put into observation cages and kept under a relatively uniform set of conditions. The total number of flies disabled after 10 min. is calculated to the percentage used and termed the "knock-down" percentage, but the insecticide is rated by the percentage apparently dead after 24 hr.

The Peet-Grady Test was adopted in 1932 by the American National Association of Insecticide and Disinfectant Manufacturers as the standard method for testing fly sprays. It was early noted that, whereas a given laboratory could reproduce its evaluations with fair accuracy, there was considerable difference between the results of different laboratories. A conjoint investigation was undertaken to ascertain the measure of agreement, and, to find out if the discrepancies could be overcome by employing a common standard insecticide, in the first instance benzophenone, for purposes of comparison. Campbell (1938) analysed the results obtained in this co-operative effort. It is not a matter of surprise that large discrepancies should have resulted when the comparative evaluations were based on the average percentage of flies killed, as the technique of testing and the methods of rearing the flies, and to some extent the equipment, varied from laboratory to laboratory. Nor did the standard insecticide proposed help in clearing up the difficulties; it was finally discarded on Campbell's suggestion for one prepared from pyrethrum. Campbell considered that efforts should be made to reduce variations of results within laboratories as much as possible, but he considered that a mode of comparative assessment and of expressing results proposed by Simanton (1937) was a feasible one. This involves ten paired tests of the standard and of the insecticide to be compared with it. In the trials the standard insecticide should give between 30–70% mortalities, if possible between 50–60%. In a series of randomized tests the mean difference between the kill obtained with the unknown insecticide and the standard should be determined, together with the

[1] Through one of these the flies are introduced.

standard error of the mean of ten differences as a measure of variability; if the latter value is less than 3 the test is regarded as having been properly conducted. The mean difference gives the grade. These proposals have been accepted by the National Association of Insecticide and Disinfectant Manufacturers of the United States, who have issued in considerable detail the procedure to be adopted in rearing the test flies and for carrying out the tests, assessing and reporting the results (see *Soap Blue Book*, 1938, pp. 145, 147, 153).

H. H. Richardson (1931) also describes a chamber method. He uses a rectangular box ($2\frac{1}{2} \times 2\frac{1}{2} \times 4$ ft.) with glass top and sides, in which a movable copper wire screen cage ($1\frac{1}{2} \times 2\frac{1}{2} \times 1\frac{1}{2}$ ft.) is placed. He incorporates a temperature control and also a fan opposite the atomizer, which is centrally fixed and projects into the test box, so that the spray is atomized into the cage, which is open at the bottom and standing on heavy wrapping paper. The fan is switched on, 40–60 flies reared under constant conditions introduced into the cage and the spray fluid (1·6 c.c.) atomized. At 30 sec. intervals, counts of the number of paralysed flies are made until well over 50% of the flies are down. After 8 min., the flies are counted and transferred to a special cage. The speed of paralytic action is taken as the time required to paralyse 50% of the flies, and it was found in the case of kerosene-pyrethrum sprays that this varied directly with their strength and was a very sensitive record of it. The death curve, obtained by plotting the percentage of the insects killed in 24 hr. against the concentration, was less sensitive.

Potter & Hocking (this number, p. 348), have studied the concentration of oil insecticides in a spray chamber, of a type similar to that of Richardson, in successive intervals of time. They find that it depends upon the rate at which the fluid is atomized into the chamber, the slower the rate at a given pressure the higher the concentration, and that it falls off rapidly at the beginning and more and more slowly with time. They also find that, with a given rate and pressure of atomization, the initial concentration is higher with the lighter than with the heavier oils, owing to the formation of smaller droplets, but other effects, such as evaporation, affect the concentration of the insecticide.

Lethal-chamber methods, although corresponding more closely than any other with actual large-scale conditions in so far as fly-sprays are concerned, are open to the objection that little is known about the distribution of either the insecticide droplets or of the insects inside the chamber. It would appear to be necessary to run a series of tests with a standard insecticide along with those of the unknown, if the results are to be susceptible of quantitative interpretation. They are also only available for such flying insects as houseflies.

Campbell & Sullivan (1938), Richardson (1932), Simanton & Miller (1938) have laid down in some detail the methods they employed for rearing and handling houseflies for spraying purposes. The age of the insect used is of importance, but Simanton & Miller (1937) have shown that with houseflies there is little difference in susceptibility between 4, 5 and 6 days old insects. Tuma (1938) has shown that resistance of cockroaches (*B. germanica*) to pyrethrum and aliphatic thiocyanates increases up to an age of 17 weeks, after which a decline follows. Other factors of importance may be length of time elapsing between feeding and spraying, the degree of crowding of a culture and the amount and intensity of illumination. Further, Murray (1937, 1938) and Miller & Simanton (1938) have demonstrated the greater susceptibilities of the

male over female houseflies to sprays and the importance of the determination of the sex ratio of the samples used in the tests. The readiest way to overcome the difficulty is to obtain 50 % approximately of each sex by preserving the inherent sex ratio of the reared cultures and using all the flies in each cage as a test unit.

Dipping methods.

These methods have been used over a considerable time; for example, Fryer *et al.* (1923) employed this technique in their investigations of derris as an insecticide. Shepard & Richardson (1931) described a dipping method which they used for testing nicotine against *Aphis rumicis*. They determined toxicity curves for the two alternatives, (*a*) where the concentration is variable and time of immersion constant and (*b*) where time of immersion is variable and concentration constant. They considered that, since in their method a wetter which might have a toxic action was not used, it had advantages over spraying technique where adequate wetting must be secured. Fleming & Baker (1934) also preferred a dipping technique for their work on the effect of contact insecticides on the Japanese beetle (*Popillia japonica*). The coefficient of effectiveness was obtained by dividing the median lethal concentration, after submersion for 120 sec., of the standard by that of the test material. A dipping method for testing egg-killing washes is also described by Kearns & Martin (1936) and by Steer (1938).

A careful study of this technique as applied to adult insects and their reaction to derris preparations has been made by Craufurd-Benson (1938) in which he examines the various factors involved, such as the age of the insects, the temperature and humidity conditions under which they are reared, the period of starvation before dipping, the time of immersion and the temperature of the bath. He evolved an improved immersion method which gives reproducible results of considerable accuracy with the particular test insect chosen. His apparatus consists essentially of a copper water-jacketed thermostat of a special type, the inner bath of which is kept at constant temperature by an electric immersion heater and stirred with an electrically driven paddle. The insecticide liquid held in a small glass beaker is placed in the inner bath until the temperature required is reached, and the insects in muslin-capped tubes are dipped in it for a definite period of time. After dipping, the tube is drained and dried by a constant procedure and the insects set aside in specimen tubes to be examined after 24 hr. The percentage numbers of insects killed for each concentration are determined. The conditions in Craufurd-Benson's experiments were: (*a*) insect, *Ahasverus advena*, (*b*) that the age of the insect should be 10–20 days, (*c*) food, mouldy rolled oats and dried yeast, (*d*) the temperature throughout breeding, and before, during and after the experiment, 25° C., (*e*) the humidity, 75% R.H., (*f*) immersion period, 4 min., (*g*) starvation period of insect, 24 hr., (*h*) observation period, 24 hr.

The most valid objection to the use of dipping methods appears to be that there is a risk of stomach poison effects being added to those given by contact. Craufurd-Benson considers this risk outweighed by other advantages.

Micropipette drop method.

This method is as far as possible free from the last objection. O'Kane *et al.* (1933) applied droplets, weighing 2 to 3 mg. each, to various areas of the last instar of the mealworm (*Tenebrio molitor*). Nelson *et al.* (1934) elaborated the method. Sixty or seventy

house flies are cooled in an electric refrigerator at $-1°$ C. (30° F.) until quiescent. A certain number are selected for uniformity of size and age, turned on their backs on a marble slab, 0·75 mm.^{3} of an alcoholic solution of the insecticide (or 9 parts ethyl alcohol plus 1 part of the liquid insecticide) is placed by means of a capillary pipette on the centre of the ventral surface of the thorax of each of the insects, while still inactive. They are afterwards placed in covered dishes with a supply of food and observed after 24 hr. They are then listed in categories of active, moribund and dead. The results given by different concentrations can be compared with those given by a standard insecticide. The flies should be reared under a constant set of conditions and preferably separated by sex after chilling. Accuracy, small amount and cost of equipment and adaptability of the method to other insects are claimed as advantages; but the authors state its disadvantages to be, that it is not comparable with field conditions and requires a considerable degree of skill.

Dusting methods.

The methods used for testing the contact insecticidal values for dusts have not been worked out in such great detail as those for testing liquid contact sprays or for stomach-poison dusts. The difficulties involved are (*a*) the difference in the rate of deposition and in the distribution of particles of different size, (*b*) difficulty in dilution, (*c*) the possibility of the separation of the diluent by air-flotation, a difficulty which may possibly be overcome in the case of vegetable insecticides by the use of organic diluents (e.g. powdered walnut shell), (*d*) the aggregation of particles during the procedure of dusting.

Trappmann & Nitsche (1934) and Thalenhorst (1937) have adopted and simplified a method of Görnitz (1933). Thalenhorst used a glass bell-jar placed over a box with sliding lid. The box contained a thick sheet of paper (area 150 sq. cm.) resting on a scale-pan, attached by a rod through a slit in the box side to one arm of a balance. The larvae to be dusted were placed in dishes in the box. With the lid closed, dust was blown into the bell-jar, the lid was then opened, the dust settled on the paper and on the larvae. When a predetermined amount of dust had fallen on the paper the lid was closed and the larvae taken out and placed on cabbage leaves. Care as to breeding and size of the insects has to be taken if accurate and reproducible measurements are to be made.

(*b*) *Stomach poisons*

In the case of contact insecticides it is assumed, if the spray or dust is evenly distributed, that the amount coming into contact with insect test-subjects of the same species and stage of development is proportional to their size, and that the amount absorbed is proportional to the concentration of the poison. For stomach poisons special precautions have to be taken if the effect produced is to be correlated with the dose administered. Much recent work has been devoted to securing this end. Special difficulties are met with when, as in the case of vegetable poisons, e.g. nicotine and derris, the material has both a contact and stomach-poison effect, and methods available for testing mineral poisons such as the arsenates might give inaccurate and misleading results unless special precautions were taken. Thus Janisch (1926) proposed feeding the larvae of *Pieris brassicae* on leaves weighed before and after dusting, the outlines of which were traced on squared paper before and after feeding. In this way the amount of leaf and poison consumed could be determined. This pioneer method

would fail to separate the contact and stomach-poison effects if the insect were allowed to move over the surface of the dusted leaf, and the same criticism would also be valid for many methods involving the use of poisoned baits. The toxicity data obtained would represent the total insecticidal effects, but there would be no means of quantitatively assessing the significance of each of them. In the case of nicotine, respiratory toxic action in the gaseous phase, in addition, might well play an important part. The problem, therefore, of differentiating the two effects is not an easy one; injection methods are too remote from practical utility and too slow in operation to permit of ready use for assessment purposes, and the ruling out of some contact action at the mouth parts seems not only impracticable but an unnecessary refinement for anything but critical physiological work.

Drop method.

Price (1920) administered known quantities of arsenical solutions to individual insects; Campbell (1926 *a*, *c*) developed and refined the technique to make it one of considerable precision. In his earlier experiments (1926 *c*) he placed a drop, containing a known concentration of poison, from a weighing burette on a leaf surface in the feeding path of the caterpillar (previously weighed); when the poison was consumed, the burette was reweighed. This procedure was repeated with other caterpillars and he determined the survival time (hence its reciprocal "the speed of toxic action") for the weight of poison consumed per gram body weight of insect. He was thus able to ascertain the relative potencies of arsenite to arsenate, and to show that insects differ markedly in their susceptibility to arsenical poisons. The method would appear too tedious and laborious for general application and later Campbell (1926 *a*, *b*) designed a microburette technique by means of which measured droplets could be placed upon the mouth parts of silkworms of known weight. The speed of toxic action for each concentration of poison administered was determined. Goetze (1932) also developed a micropipette method for comparing pyrethrum with the arsenicals. The poisons were diluted with honey solution (2 : 1), taken up in the graduated pipette, which was laid on its side. The tip of the pipette passed through a hole of a cage. Bees were used as test insects, the amount taken being measured and the toxic effect recorded.

Leaf-sandwich method.

Another technique, known as the leaf-sandwich method, was devised by Campbell & Filmer (1929); it is a great improvement on Janisch's leaf method. It is primarily applicable to dusts, but there is no obvious reason why it should not be adapted to spray fluids. Essentially, the technique consists of blowing a quantity of the poison dust into an inverted bell-jar through a tube fitted with a ball-valve. After a short period, during which the larger particles are deposited, the jar is moved on to a plate on which are distributed circles of leaf and a number of circular glass cover-slips of known area. The cover-slips are weighed to determine the amount of the deposit, the leaf circles are made into sandwiches by superimposing other leaf circles of the same size and are fed to individual caterpillars, the weights of which are known. After feeding, the unconsumed area is determined either by means of a planimeter, cross-section paper or by photoelectric cell (Bulger, 1935). Stellwaag (1931) suggested some simplification of the process and coated the leaf disks with starch paste. Görnitz (1933) introduced a method for directly weighing the deposit, by means of a frame inserted into the dusting chamber and attached by means of a bar to one arm of a balance;

thus predetermined weights of poison can be deposited. Bulger (1937) has also suggested means for controlling the amount of deposit consumed.

By this method much important toxicological data can be accumulated. The speed of paralytic action (100 divided by the active period in hours) and the speed of toxic action (100 divided by the survival time) can be determined for each dose consumed. In addition, the proportional number of deaths can be ascertained for the amounts of poison consumed. The latter values enable a table to be constructed indicating the sublethal, intermediate and lethal doses. The chief defect, according to Campbell & Filmer (1929), of any leaf-area method is that the doses cannot be accurately predetermined and they consider that the numbers of larvae in the sublethal and lethal zones are wasted, and that time and insects are thus thrown away, since they do not help in delimiting the medium lethal dose (that dose which kills 50%). This dose occurs in the intermediate zone of toxicity and can be approximately determined from a consideration of the data accumulated within that zone. The modifications proposed by Bulger (1937), by Görnitz (1933) and by Thalenhorst (1937) may have eliminated some of the waste of insects.[1]

Campbell (1930), however, has pointed out that the median lethal dose does not present a complete picture of the effectiveness of a stomach poison, since the time factor involved in death or recovery is unstated. Indeed, Campbell & Filmer (1929) consider that the effectiveness of a stomach poison should be clearly differentiated from toxicity; the latter term implies killing power, while the former, in addition, is influenced by the rate or speed of toxic action and by the cumulative deterrent effects upon further feeding. Campbell (1930) illustrated the necessity of close attention to the factors determining relative toxicities by plotting against the dosage (mg. per g. body weight) the speed of toxic action, as represented by the reciprocal of the active period and as represented by the reciprocal of the survival period, for two different poisons (sodium silicofluoride and acid lead arsenate). He determined the areas between each curve, the ratio of which gives the relative toxicity over the whole dosage range. He pointed out that, since upon the onset of paralysis no further feeding takes place, the determination of the active period of the insect may be a better assessment of relative toxicity than that of the survival period. In such a case the terms relative toxicity and relative effectiveness would appear to merge into one meaning.

Bulger (1937) has pointed out that the median lethal dose of an insecticide dust may depend partly on the size of particle. In the sandwich method only the smaller particles are utilized. It would thus appear that, for purposes of determining the relative potencies of more than one sample, the particle size should be of comparable dimensions, a requirement which, for compounds of widely different density, may not be easy to attain.

Cage method.

This method, in which several insects are confined in each cage with treated foliage, is not, as generally used, capable of giving an evaluation of the stomach-poison action alone. It affords an indication of total toxic action and deterrency. It was used by Gimingham & Tattersfield (1928) for some preliminary work on the reaction of

[1] Bulger (1932) mentions another difficulty inherent in the method, namely, the possible separation of diluent or adjuvant from the poison in the process of settling, necessitating separate applications of the components.

mandibulate insects to foliage sprayed with extracts of certain leguminous fish-poisons, and is the only method available in special cases, such as that of exploring the toxic action of chemicals to shy-feeders such as the Japanese beetle (*Popillia japonica*), for which specially constructed and illuminated cages were used by Fleming (1934). He demonstrated the great importance of the effect of temperature, humidity and degree of illumination on the susceptibility of this insect.

Plug method.

This method is primarily used for testing insecticides against codling-moth larvae, and by its nature it measures the total toxic effect due to both stomach and contact action, if the latter is shown by the compound. Newcomer (1926) sprayed apples upon which newly hatched larvae were placed and the number of worm entrance-holes and stings determined. Siegler & Munger (1933) improved this technique by taking cylindrical plugs of de-cored apples, inserting the plugs into vials with the skin, which must be unblemished, uppermost, spraying uniformly, pushing the core home in the tube after drying, sealing the edge of the apple with wax and lead resinate and waxing the raw end. The tube is now ready to receive the codling-moth egg after which it is sealed. The tubes are set aside and examined later for the number of entrances, mortalities and stings, which can then be correlated with the concentration of poison used.

(*c*) *Fumigation experiments*

Bovingdon (1934) has reviewed the difficulties met with in this type of work. They are (*a*) the loss of gas from the fumigation chamber due to the introduction and removal of the insects, (*b*) the provision of means for ensuring uniform concentrations of the poisonous gas in the fumigation chamber by thorough mixing, (*c*) the determination of the gas concentration during an experiment, (*d*) the preparation of a gas mixture of a predetermined composition. He reviews the literature of the subject, gives brief descriptive summaries of the apparatus used by other workers in this field, and an account of a new type of apparatus for fumigating insects and the experimental procedure necessary for its use. The apparatus, which is of a somewhat complicated type, is housed in a constant temperature cabinet. By its use Bovingdon has in a very large measure overcome the difficulties mentioned above. The technique employed assures thorough mixing and circulation of the gas mixture and also secures that the test insects are subjected to the full concentration of the fumigant gas from the start of the experiment. The gas concentration in the fumigation flask is practically unaltered by the processes of introducing and removing the insects. If any criticism were to be offered on this ingenious apparatus it would concern its somewhat complicated nature and the rather involved technique requisite for its correct use.

Gough (1938 *a*) describes a modified form of the same apparatus in which Bovingdon and he have introduced a number of simplifications. The rocking of the apparatus provides for both circulation and mixing of the gas mixture. Gough also employed in his work a technique of a very simple type for circulating a fumigant-air mixture of known composition over insects, which to a great extent overcomes many of the difficulties met with in this work. The cleaning of the apparatus is easier, there are fewer stop-cocks the greasing of which may absorb some of the fumigant, the rubber-tubing exposed to the gas is reduced to a minimum and the exposure of the insects to mercury, which Gough (1938 *b*) has shown to have a toxic effect, may be largely avoided. The apparatus as so far developed does not permit, as in Bovingdon's

original type, of the insects being subjected to a full concentration of fumigant at the commencement of the experiment, and there may be a slight loss of fumigant on the introduction of the insects into the fumigation chamber. These difficulties do not seem insuperable, and owing to the large volume of the gas reservoir the proportional losses of fumigant due to the presence of the insects is minimized and accurate dosing is apparently rendered more easy.

Peters & Ganter (1935) and Peters (1936) describe a relatively simple apparatus, in which a series of fumigation chambers are arranged in parallel round a common gas distributor in a thermostat, humidity being kept constant. The amount of rubber-tubing and the number of corks exposed to the action of the gas would appear to be a drawback to its use. When the concentration-time relationships were examined, Haber's formula $c \times t = w$, which in effect states that toxic action is a product of concentration and time, was found approximately to fit the data obtained, a result in agreement with those of Mayer (1934) using ethylene oxide. A divergence from this equation arises apparently when low concentrations are used and at short exposures to high doses.

In general, for cases where exposures are given for definite times, after which the insect is taken from the apparatus and examined after a period of time in order to ascertain the effect, the formula $c^n t = w$ has been found to fit these types of data more closely. n usually varies between 1 and 2 and Haber's formula would appear to be a special case.

Busvine (1938) has reviewed and experimentally examined the factors affecting the action of fumigants and surveys the precautions to be taken in the laboratory assessment of their toxicity. He shows that different species of insects have a different order of resistance to different fumigants, that without adequate mixing and circulation of the gas mixture, considerable errors in determining the concentration of the fumigant may occur, and that despite great precautions in subjecting the insects rapidly to the action of the fumigant, there is a time lag before full concentration is attained, which, on the assumption of concentration × time being constant, he was able to calculate as a complete hiatus of 1·6 min. for his experimental conditions. The effect of temperature was a complicated one; physical properties such as vapour pressure, diffusion and sorption of the fumigant may play an important part. Toxicity is at the same time closely bound up with the physiological processes of the insect; rise in temperature as it increases metabolism can be correlated with increased susceptibility. The bearing of other variable factors on physiological condition and insect resistance are tabulated, and it is shown that, in general, factors increasing the rate of metabolic processes (as judged by respiration) increase susceptibility. Starvation, however, which lowers respiration rate, appears to decrease susceptibility to ethylene oxide, but to increase it to hydrogen cyanide.

In addition to dose-mortality relationships which are examined by the method of probits, Busvine analyses time-concentration data and finds that the logarithms of the times taken to kill 50 and 99% of four different insects bears a linear relationship to the logarithms of the concentrations used. He claims that the formula $c^n t = w$ (where c = concentration in mg./l. N.T.P., t = time, and n represents the slope of the log c log t regression line) gives a satisfactory representation of concentration-time curves for the four insects used as test subjects, a practical implication being that two exposure times[1] need only be investigated for its solution. He suggests the use of this formula for

[1] In the present state of our knowledge, it would appear advisable to have more than two times in order to verify that Busvine's formula fits the data.

expressing resistance of insects to a particular fumigant, the criteria requisite being concentrations which in a 5 hr. exposure secure 50 and 99% kill, and the value of n. The valid use of this formula would appear to necessitate the determination of probit-log concentration regression lines, since kills of 99% cannot be determined experimentally with any accuracy and are generally obtained by extrapolation. These lines should be straight and parallel over the range of exposure times. From Busvine's data one would draw the conclusion that his probit-log concentration regression lines do depart from parallelism. The net effect of this lack of parallelism on the determination of n for 50% kill does not appear to have been considerable, but the suggestion of applying the same constant to the 99% mortality values seems a highly doubtful one. Indeed, Bliss & Broadbent (1935) found a similar formula to fit their data for hydrocyanic gas as a fumigant for *Drosophila*, but noted that it changed from $c^{1\cdot9}t = 10\cdot2$ for a 50% mortality to $c^{1\cdot3}t = 26\cdot3$ for 97·5%, and they emphasize the importance of the level of mortality used in determining formulae of this type which have been somewhat generally used in toxicological work. It is perhaps advisable to remember that these curves, although they may be convenient for purposes of comparing relative rates of action, e.g. of drugs, appear to have provided singularly little information regarding the fundamental nature of their action (Clark, 1937).

Strand (1930), Lindgren & Shepard (1932), Shepard & Lindgren (1934) and Shepard *et al.* (1937) used a much simpler form of apparatus than those outlined above. They examined the mode of comparative assessment of insect fumigants. Their dosage-mortality curves are of the same sigmoid type as those obtained by workers engaged in the examination of the effect of sprays. The 50% mortality point or the median lethal dose is regarded as preferable for comparison, since it is more reproducible, but Shepard & Lindgren (1934) point out that, since the respective curves are not always parallel, a comparison of values near the 100% mortality, although only approximate, may be of greater practical value. Shepard (1934) suggests the use of the formula

$$x = K + k.\log \frac{y}{100 - y}$$

for obtaining an estimate of the 99% lethal dose, where x and y are the values for dosage and mortality respectively, K the concentration required to produce 50% mortality and k the difference in the concentrations giving 90 and 50% mortalities.

Shepard *et al.* (1937) emphasize that a serious source of variation in results lies in the difficulty of distinguishing a sharp end-point, and that the latter should be so chosen that the estimated mortality will represent the eventual result for each species of insect as nearly as possible. They stress the importance of allowing for the recovery of resistant species as well as for tardy mortality induced by slow-acting chemicals.

Two methods for determining the toxicity of nicotine in the vapour phase are described by C. H. Richardson & Haas (1932) and by H. H. Richardson & Busbey (1937).

Gough's work (1938 *a*, *b*) indicates that care is not only needed in rearing test insects in a constant way, but that there is a marked effect on resistance to hydrocyanic acid gas of the time elapsing between the removal of certain insects from their food supply (in his case adults of *Tribolium confusum* in flour) and their fumigation. Those fumigated shortly after separation were less resistant than those separated for longer periods, males being less affected than females by early removal from flour. He also noted the emanation of a toxic substance from *Tribolium* adults, a result which

affected the type of container in which these insects could be placed for the purpose of fumigation. He observed cumulative losses of hydrocyanic acid gas in apparatus used previously for fumigating the adults, and the substance causing this loss could not be removed by evacuation. In addition, this worker's observation of the toxicity of mercury vapour to both the eggs of the bed bug and the confused flour beetle would indicate that exposure to this metal should be reduced to a minimum or, as Bovingdon suggests, gold leaf should be employed in the circuit to provide for its elimination from the fumigation chamber.

A CONSIDERATION OF SOME OF THE METHODS OF ASSESSING RESULTS

The toxic action of an insecticide may be quantitatively judged in three ways: (*a*) by the effect produced by different concentrations in a given time, (*b*) by the effect produced at different intervals of time, the concentrations being kept constant, (*c*) by the effects produced at different intervals of time by different concentrations. When plotted (*a*) gives rise to what are known as dosage-mortality curves, (*b*) to time-mortality curves, and (*c*) to time-concentration curves.

The curves produced are rarely linear and our knowledge of their nature and their interpretation has been greatly increased in recent years by the work of Osterhout (1922), Fisher (1924), Henderson-Smith (1921, 1923), Trevan (1927), Clark (1933, 1937), O'Kane *et al.* (1930, 1934), Hemmingsen (1933), Gaddum (1933), Bateman (1933), Bliss (1935 *a*, *b*, 1937) and others, for a full appreciation of which the original papers should be seen.

(*a*) *Dosage-mortality curves*

In general, it may be stated that the investigator of the insecticidal potency is mainly concerned with the ultimate death or recovery of the organism after the administration of the poison. Frequently, however, it is not possible to divide treated insects into two sharp divisions, those killed and those not affected. There are often gradations of toxic effect, the apparently dead, the moribund, those badly affected, slightly affected and those not affected, with graduations within each of these categories. It is necessary, therefore, to keep the treated insects under observation for a sufficient length of time in order to ascertain with some degree of certainty the ultimate effect, and, to have experience of the course narcosis may take. For example, in the case of pyrethrum an insect may recover from deep narcosis, whereas, after treatment with the rotenone-containing insecticides an insect, showing only partial paralysis, will gradually sink into a moribund condition. It is advisable for critical work, therefore, to fix upon certain symptoms as an aid to classification, and either to assess toxicity on the numbers apparently dead, or to group together the seriously affected insects, or to award marks for the different categories into which the treated insects group themselves. All these methods have been used with success. In the system of marking, so far, only arbitrary marks have been used (by Fryer *et al.* 1923 and by Worsley, 1934), but since it might well depend for its validity on the physiological action of the particular poison, a method of awarding marks based upon a statistical analysis of the data for each poison would appear to be called for.

The practical man wishes to know that concentration which will kill 100% of his organisms. Unfortunately, this is unattainable. When the concentrations used are plotted against the percentage kill or the percentage effect, as measured by some particular physiological action after a sufficient time has elapsed for a complete

response, the resulting curve is usually sigmoid or S-shaped, that is, it tails off at its upper and lower ends, where it approaches zero and 100% mortality, the change in percentage kill per unit of dose or concentration being greatest near the 50% kill. Fisher (1924) and Trevan (1927) have pointed out that for several different compounds the best point on these curves for purposes of comparison is at the concentration or dose securing 50% kill, which Trevan has named the *median lethal dose* or L.D. 50.

Although there has been discussion on the interpretation of these curves (see Clark, 1933), they are considered as being an expression of the variation in susceptibility between individual organisms drawn at random from the population. Were one able to express quantitatively the distribution of this susceptibility among a number of organisms the curve expected would be a normal frequency distribution (bell-shaped)—the S-shaped curve being its integrated or cumulative form. It has, however, been found that the susceptibilities are symmetrically distributed when the scale of dosages is logarithmically rather than arithmetically expressed.

It was independently observed by Bliss (1934), O'Kane *et al.* (1934), Gaddum (1933) and Hemmingsen (1933) that if the percentage of insects killed for several concentrations of a poison were equated to theoretical dosages, inferred from the cumulative normal curve, and expressed in units of standard deviations, and these units were plotted against the logarithms of the concentrations, a straight line resulted. These units have been termed "Normal Equivalent Deviations (N.E.D.)" by Gaddum, and "Probits" by Bliss. In Gaddum's terminology they are positive or negative, depending on whether the percentages are above or below 50%, which is 0. For ease of analysis, Bliss adds 5 units to each N.E.D. and thus gives them all positive values. Bliss (1935 *a*, *b*) has given much time to a consideration of this transformation, has published tables of probits, and of weights to be given to their values, since they are not uniform in this respect, the weight to be ascribed being a maximum at probit 5 (=50% kill) and falling off towards the values for 0 and 100% kills. Bliss (1935 *a*, *b*) in his papers gives detailed examples of the use and the working-up of data by this method. The means of determining relative potencies of insecticides and drugs by its aid are given by Bliss (1935 *b*), by Irwin (1937) and, of certain insecticides, by Cochran (1938). For the essential tables and further refinements developed, *Statistical Tables for Biological, Agricultural and Medical Research*, by Fisher & Yates (1938), should be seen.

This linear transformation has greatly facilitated the study of insecticidal action. It has made possible the fuller use of the data accumulated for the purpose of comparing toxicities, for, instead of having to rely upon one point on the curve, nearly all the points can be pooled and brought into use for purposes of assessment. It enables one to give an approximation to the concentration giving 100% effect and, by means of the χ^2 test, to determine the homogeneity of the data and goodness of fit of the regression line and to eliminate doubtful determinations. It is also possible to calculate the error in position and slope of the regression line. This technique, in short, gives an expression of the significance of the results.

(*b*) *Time-mortality curves*

Time-mortality data have been defined by Bliss (1937) as records of reaction time, which can be reduced to a form showing the number of organisms reacting to a toxicological stimulus in each of several successive periods of time. It was shown by

Henderson-Smith (1921, 1923) that such curves (usually S-shaped in their cumulative form) are, as in the case of dosage-mortality curves, explicable on the assumption that individual organisms differ in resistance and that the distribution in resistance grades approaches the normal frequency curve. These curves have considerable usefulness in giving information on toxicological problems (e.g. in fumigation), although it is generally agreed that for most practical purposes the dosage-mortality curve is more informative. If, for example, one were to assess the comparative toxicities of pyrethrum and derris by determining the time at which a number of insects were paralysed, one might arrive at a wholly erroneous estimate of their relative values as insecticides, since one is rapid in action with a tendency towards recovery, and the other slow in action with a tendency towards deepening paralysis. Bliss, who has examined statistically time-reaction curves, considers that they are often of indeterminate value unless they can be transformed to straight lines by conversion of percentages to probits and the observed time to logarithms or rates.

(c) *Time-concentration curves*

These have been often used in toxicological work. As frequently expressed, they result in the replication on the same diagram of curves giving the relationship between time and percentage of organisms killed or surviving for a number of concentrations, and are commonly used to determine the rate of toxic action. This can readily be done by taking the reciprocals of the time required to produce the same effect. If the toxic effects for the various concentrations are determined after a number of different periods and complete curves are drawn, no difficulty is likely to be met with in determining the rates of action. If this is not possible then a careful choice of procedure is needed. Henderson-Smith (1923) and Osterhout (1922) have pointed out that the rate of action can be regarded from two points of view, (*a*) the toxic effect produced in a given time, (*b*) the time taken to produce a given toxic effect. In biological reactions the rate rarely proceeds at uniform speed and as determined by the first of these alternatives may lead to serious errors of assessment. This can be clearly seen if two unimolecular reactions, one proceeding at twice the rate of the other, are plotted. The same generalization can be drawn with respect to the determination of temperature coefficients as they affect rate of toxic action. Henderson-Smith's investigation (1921) on the toxicity of phenol to *Botrytis* spores shows also that the shape of the curves in which percentage kill is plotted against time may alter with concentration, and thus the ratios of the time taken to kill varying proportions, e.g. 25, 50, 75%, for several concentrations of poison lead to different results. Since, however, the greatest uniformity in the rate of response of a population of organisms is found in the region of the 50% effect, this point is the best one for assessing rate of toxic action.

Campbell (1930) considered that the most nearly complete picture of the acute toxicity of a compound would be obtained by plotting the reciprocals of the recovery periods and of the survival periods against doses covering as wide a range as possible. This method would give a graph resembling the cross-section of a valley, one side representing the effect of sublethal doses, the other representing the effect of lethal doses and the bottom of the valley representing the region of the median lethal dose. For comparing insecticides, however, he plotted the speed or rate of toxic action (i.e. the reciprocal of the time taken to paralyse or to kill) against the dosage. Such curves, often of a sigmoid character, were not regarded as owing their character to variations

in individual susceptibility (1926 *a*). By measuring the area below the curves given by the respective compounds he assessed relative toxicity.

Bliss (1936) examined in detail some of Campbell's data (1926 *a*, *b*) showing the change of rate of toxic action with oral dosage of arsenic when silkworms were used as a test subject. Several important points were brought to light. In oral experiments, the dosage is most commonly represented as the weight of poison administered per body weight; this practice was examined by Bliss. His analysis showed that the rate of toxic action seemed to be a linear function of the log of the dose per larva and the log of the body weight. The power of the body weight W^h, however, which eliminated the effect of body size upon the rate of toxic action, depended for its magnitude upon whether the speed of toxic action or its log were used in computation. In the former case h increased from 1·67 to 1·96 as one passed from the 2nd to the 5th instars, but when the log rate was used h was the same for all instars and averaged 1·5. Thus for all instars of the silkworm, when the log rate of toxic action was plotted against the adjusted dosage, $\frac{\text{mg. arsenic}}{(\text{g. body weight})^{1\cdot5}}$, expressed in logarithms, a straight line resulted. If the separate instars were taken and the rate of toxic action was plotted against the log dose, $\frac{\text{mg. arsenic}}{(\text{g. body weight})^{1\cdot67-1\cdot96}}$, straight lines were also obtained for each instar. If it could be assumed in these cases that poisoning followed the same course at both low and high dosages, and therefore, that the individual rates of toxic action would be symmetrically distributed about the curves throughout their course, theoretically it should follow, that the point at which each straight line intersects the base line should represent the median lethal dose for the particular instar. Such a deduction, however, requires experimental confirmation.

Field experiments

Until recent years the usual method of assessing results of insecticidal trials in the field was by visual inspection. Now, with the increasing use of appropriately designed experiments, statistical analysis is yielding quantitative data which give a measure of the significance of the results obtained. The aim of the experiment may be to ascertain the effect upon crop yield or an estimate of the degree of control of the pest, involving counts of the latter, or a combination of the two methods. The experiment may take the form of the randomized block or of the Latin square, with the subsequent use of the analysis of variance technique for the determination of the significance of the results. Bartlett (1936) has pointed out, however, that if direct counts of pests are to be made, a careful examination of the suitability of the design may be needed. The statistical analysis may also require special treatment.[1] It is outside the scope of this paper to deal with this complex subject, but it should be indicated that before field experiments are carried out on the control of insect pests by insecticides, the experiment should be soundly designed from a statistical point of view in order to yield a maximum of information. Two recent examples of the use of the Latin square technique are given by Gaines (1937 *a*, *b*). An experiment, arranged in randomized blocks, in which derris and nicotine were tested for the control of raspberry beetle (*Byturus tomentosus*), was carried out by Steer (1933) and yielded results of considerable economic importance.

[1] See also Cochran, W. G., "Some difficulties in statistical analysis", *Emp. J. exp. Agric.* (1938), **6**, 157–75.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Annual General Meeting of the Association of Applied Biologists held in the Imperial College of Science and Technology, London, on Friday, 10 February 1939. The morning session began at 11.45 a.m. in the Botany Lecture Theatre, and the afternoon session at 2.30 p.m. in the Metallurgy Lecture Theatre. The Chair was taken by the President, Mr C. T. Gimingham.

Discussion on Sugar Beet Problems

The following papers were read:

I. Acidity and manganese deficiency problems in connexion with sugar beet growing. By W. Morley Davies, M.A., B.Sc.

II. The effect of boron on the growth and quality of sugar beet. By A. W. Greenhill, Ph.D., M.Sc., F.I.C., A.R.C.S.

III. Sugar beet pests. By F. R. Petherbridge, M.A.

IV. Pests of the sugar beet crop in the Midlands. By A. Roebuck, N.D.A.

V. Sugar beet diseases. By H. H. Stirrup, M.Sc.

I. ACIDITY AND MANGANESE DEFICIENCY PROBLEMS IN CONNEXION WITH SUGAR BEET GROWING

By W. Morley Davies, M.A., B.Sc.

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Acidity

Sugar beet may be regarded as one of the acid sensitive crops and falling into the same class as barley, clover and mangolds in this respect. In the earlier days of beet growing in this country acidity doubtless accounted for at least 75% of the failures of the crop. Gradually, however, farmers have come to realize that the presence of non-acid conditions are essential and nowadays it is the exception to find many failures from this cause. Being a deep-rooting plant and carrying on some of its feeding in the subsoil, adequate preparation in the way of liming an acid soil should be made some time before the crop is grown. Many growers think that they have done sufficient if they give a dressing of lime after ploughing—lightly cultivating it into the surface—and numerous cases of this practice have come to the writer's notice. Under these conditions short stubby plants are obtained with innumerable side rootlets giving a

hairy appearance. It is essential that the lime should be applied two or three years previously, if the soil is at all acid, so that it will have an opportunity to penetrate to the subsoil.

Under acid soil conditions the seedling usually grows fairly satisfactorily as far as the fourth leaf stage. Thereafter, the plant remains stunted throughout the season or dies. The small plants have a rather characteristic dark green appearance and often develop a red colour at the base of the petioles. Under still more acid conditions the seedling may not grow beyond the cotyledon stage, shrivelling in dry weather. Under less severe, but still somewhat acid conditions, the crop may grow fairly normally without, however, yielding satisfactorily. Acidity seems to have no very marked influence on the sugar percentage but reduces the weight of sugar per acre in proportion to the diminution in yield.

Soil conditions

Under excessively acid conditions the crop will fail more or less completely. This condition, however, is not often seen under farming conditions, since, in the field, acidity manifests itself by a patchy crop. The changes from favourable to unfavourable conditions are often sharply defined. The usual field symptoms are either patches where the beets are diminutive, or patches where the plant is "gappy". From the examination of numerous fields and by comparisons of good and poor growth in those fields the crop would appear to fail below a critical *p*H of about 5·3. It is interesting to note that failures at this figure have been recorded as far apart as the Midlands, the North of Scotland and Southern Ireland. Precision in stating any absolute value for such a figure is not claimed since, doubtless, it varies somewhat with different soil and climatic conditions.

The results of two experiments which have been carried out in the West Midlands can be cited to illustrate the effect of acid soil conditions on yields.

Liming experiment at Harper Adams College

In 1928 an experiment in the form of a Latin square and including four graded treatments with lime was laid down on a sandy soil. This has now been conducted over ten years without further additions of lime. In 1930 sugar beet was grown and the results for that year are given in Table I.

Table I

Dressing cwt. $CaCO_3$/acre	Yield of beet tons/acre	% sugar	*p*H	Exchangeable CaO
0	4·5	16·7	4·9	0·089
25	9·1	17·1	5·3	0·114
50	10·25	17·1	5·5	0·115
100	10·1	17·2	5·9	0·161

Lime requirement, Hutchinson & McLelland, 40 cwt./acre $CaCO_3$.

The results show that the effect of the smallest dressing of lime substantially and significantly increased the crop. Thereafter, no significant increases were produced by successive dressings. The results also show that the critical acidity value is about 5·3 and an exchangeable lime content of about 0·09% (calcium oxide). The latter figure would doubtless vary considerably on different soil types. There was no very marked relation between the sugar percentage and the base status of the soil.

Experiment using sugar beet factory waste lime

Another experiment which throws light on the effects of acidity and subsequent liming on the yield of beet and mangolds was carried out in the West Midlands. In this, sugar beet factory waste lime (sludge and dried) was tested against a standard ground limestone. The results are shown in Table II.

Table II

	Yield of crops				
	Sugar beet (1935)		Mangolds (1936)	Sugar beet (1937)	
	Roots (washed) tons/acre	Sugar cwt./acre	Roots tons/acre	Roots (washed) tons/acre	Sugar cwt./acre
Control	9·76	33·14	17·83	4·30	13·2
Half-ground limestone	11·28	38·12	20·21	7·04	22·5
Full-ground limestone	10·74	36·16	20·68	7·94	25·0
Half-dried sludge	11·31	38·14	20·52	7·47	23·7
Full-dried sludge	12·05	41·10	22·18	8·88	28·5
Half-wet sludge	11·02	37·54	20·07	6·67	21·1
Full-wet sludge	11·22	38·46	20·80	8·19	26·4
Total averages	11·03	37·52	20·33	7·21	22·9
Limed plots, averages	11·27	38·25	20·41	7·70	24·5

	Soil reaction (*p*H)			
	Initial, 1934	1935	1936	1937
Control	5·4	5·4	5·1	5·0
Half-ground limestone		5·8	5·5	5·2
Full-ground limestone		5·9	5·5	5·5
Half-dried sludge		5·8	5·5	5·3
Full-dried sludge		6·3	5·8	5·6
Half-wet sludge		5·8	5·6	5·5
Full-wet sludge		6·1	5·7	5·5

Liming with ground limestone, dried sludge and wet sludge produced marked increases in yield both at full-dressings and half-dressing rates. The increases resulting from liming were not so great as in the previously quoted experiment since the soil was initially less acid.

Manganese deficiency

Troubles associated with manganese lie on another part of the acidity scale and only occur, at any rate in this country, under conditions tending to alkalinity.

Appearance of the crop. Sugar beet and mangolds. Both crops seem sensitive to manganese deficiency and exhibit a condition referred to in beet as "Speckled Yellows". For a time, after singling, the plants appear to be quite normal, then, within a few days, a yellowing of the foliage becomes apparent. This happens when the plant is about 6 in. high and usually in the months of June or July. Closer examination of the foliage shows the presence of numerous yellowish blotches occurring in the inter-veinal tissue. By transmitted light these are particularly noticeable and stand out in comparison with the darker green of the veins. At a subsequent stage this blotched tissue dies and may or may not fall out of the leaf. It is possible that two conditions of these symptoms occur, (*a*) where the blotches just turn yellow and remain so, and (*b*) where they turn yellow, then brown, eventually dying and falling away from the

leaf. Many of the affected leaves, but not by any means all, assume a triangular shape due to the rolling of the edges towards the centre. Later in the season there is considerable recovery, the younger leaves being less noticeably yellow, and towards autumn the crop appears normal.

Soil conditions conducive to manganese deficiency. Two soil conditions seem necessary, in this country at any rate, for the production of the disease in manganese sensitive crops: (*a*) a reaction value which exceeds *p*H 6·5, and (*b*) the presence of considerable amounts of organic matter. These conditions must occur at one and the same time. Seldom do the symptoms appear in well-limed mineral soils of normal or low organic matter content, neither do they occur in soils of high organic content unless excessive liming or natural conditions give rise to a high reaction value. There should be no difficulty in recognizing soils in which the disease may appear, since they are usually black or dark grey in colour. Peat soils, and humus-sands occurring on comparatively recently reclaimed heath and moorland are the main types on which the disease occurs. Cases are known where certain fields are naturally subject to the disease. Among those cases of the occurrence of Speckled Yellows reported by Cranfield, one occurred in a field where a shallow fen soil overlaid a chalk gravel, and another where a peaty sand overlaid calcareous gravels of the Old Trent Valley. In those cases the top-soil reaction was naturally and permanently maintained above *p*H 7.

Various hypotheses have been put forward to account for the effect of alkalinity in rendering the manganese unavailable in soils rich in organic matter. Some investigators conclude that under alkaline conditions the more easily available or bivalent form passes to a less readily available condition of a higher oxide. The close association of manganese deficiency with soils rich in humus suggests that the manganese becomes tied up in some way with the organic complexes. Piper, working in Australia, found that the symptoms appeared on oats when the *p*H exceeded 6·7, especially on heavily limed black sands (sandy podsols).

The figures in Table III were obtained from three affected areas in an experimental field near Tamworth and refer to oats.

Table III. *Relation between soil conditions and Grey Speck in oats* (*July* 1936)

	Area A		Area B		Area C	
	*p*H	Exchangeable CaO %	*p*H	Exchangeable CaO %	*p*H	Exchangeable CaO %
Crop good	6·2	0·41	6·5	0·40	6·5	0·46
Crop somewhat affected	6·6	0·44	6·7	0·42	6·5	0·47
Crop severely affected	7·3	0·52	7·3	0·46	6·9	0·48

Manganese appears unique among the exchangeable bases in that it may pass from the active form into the unavailable higher oxide or vice versa, according to the balance of the oxidation-reduction equilibrium prevailing in the soil. This balance appears primarily to be determined by the soil reaction.

An interesting paper by Gerretsen of Holland provides evidence that Grey Speck of oats may not be due solely to manganese deficiency. Gerretsen believes that the disease is bacterial. Plants with abnormal metabolism due to lack of available manganese are stated to be unable to synthesize organic acids in sufficient quantity

to neutralize ammonia produced by the bacteria in the vicinity of the roots. Lack of carbohydrates in the leaves weakens them so that they bend in a characteristic manner. Vigorous plants growing under normal conditions are little affected by the bacteria. If this be the case then the symptoms associated with Grey Speck must be regarded as secondary and not primarily due to manganese deficiency.

Control of the disease

Measurements on the effect of soil treatment in the resulting crops have been made, more particularly on oats. The results of satisfactory forms of treatment are however equally applicable to sugar beet.

Under field conditions several methods of control are being investigated:

(1) Treatment with compounds containing manganese applied either to the soil as a top dressing or as a spray on the foliage, such as manganese sulphate, basic slag, etc.

(2) Introduction of acidifying conditions by treating the soil with sulphur, sulphate of ammonia, etc.

(3) Introduction of reducing conditions by incorporation of farmyard manure and chemical substances, hydroquinone, sodium sulphite, etc.

Treatment with compounds containing manganese

Of these compounds manganous sulphate seems to be the one most widely used. Experiments have also been conducted with other salts of manganese, notably the chloride. In addition, basic slag, as a source of manganese (9·3% manganous oxide), has been used with some success.

Results obtained by Morley Davies & Irons on oats, cut whilst still green, near Tamworth in Warwickshire with manganese sulphate and basic slag in 1934 and 1935 are shown in Table IV.

Table IV. *Effect of slag and manganous sulphate in the control of Grey Speck of oats*

	Yield of dry matter (cwt./acre)		Mn uptake (lb./acre)	
	1934*	1935†	1934	1935
Control	20·8	39·2	0·57	0·35
Slag (6 cwt./acre)	21·7	42·2	0·65	0·43
Slag (12 cwt./acre)	23·8	43·2	0·59	0·51
$MnSO_4$ (56 lb./acre)	27·1	47·2	0·94	0·73
$MnSO_4$ (112 lb./acre)	30·6	46·5	1·44	0·83
Significant differences are greater than	3·54	3·94	—	0·18

* Air-dried matter. † Absolute dry matter.

Stewart investigated a case of manganese deficiency in oats at Holmeswood, Lancashire, where the soil *p*H was 6·9. Treatment with manganous sulphate and basic slag resulted in increased uptake of manganese with the heavier dressings (Table V).

The effects of manganese sulphate and slag as control materials in improving the crop are corroborated by more recent observations on plots laid down by Morley Davies, Bates & Tilley near Stourbridge. Here the effect of slag was more pronounced on crops less susceptible to the disease, such as barley, than on oats, but was in no case so effective as manganese sulphate. It was interesting to note that Marsh

Spot in peas (1938) was controlled at the same centre only by dressing with manganese sulphate, other forms of treatment proving ineffective.

Table V. *The effect of dressings of manganous sulphate and slag on the uptake of manganese in oats*

Treatment	Mn_3O_4 % in dry matter of crop
1. Control	0·0021
2. Manganese sulphate 56 lb./acre	0·0020
Manganese sulphate 112 lb./acre	0·0041
3. Basic slag 5 cwt./acre	0·0018
Basic slag 10 cwt./acre	0·0034

Treatment with acidifying materials

Sulphur, incorporated with the soil, has been widely used as an agent for acidification. It reduces the soil reaction and consequently increases the supply of available manganese. Plots at Stourbridge were dressed with sulphur at the rate of 1 ton/acre and were rendered markedly more acid, the *p*H being reduced by the single dressing in 1937 from 6·8 to 5·7; this was maintained at *p*H 6·0 in 1938 without further dressing. The control of the disease in all sensitive crops was good and was doubtless due to an increase in the readily available manganese, viz. from 0·14 to 0·27 mg./100 g. soil.

Sulphate of ammonia was also tried in 1938 at the same centre, but, whether owing to the dry conditions of the season, or for some other cause, did not prove nearly so effective as sulphur.

Introduction of reducing conditions

Piper's work has shown that temporary waterlogging of plants grown in soil in pots had a marked effect on eliminating symptoms of the disease. This he attributes to the introduction of conditions which resulted in the reduction of the more highly oxidized and unavailable materials to a soluble and available state. In this country Godden & Grimmett found that oats grown in pots without drainage contained about six times as much manganese as those in pots provided with drainage, indicating that reducing conditions increased the solubility of the manganese. In practice this would be difficult to achieve except under conditions where irrigation could be controlled and, even then, might not be effective.

The effect of incorporating farmyard manure with the soil is marked by a reduction of the symptoms shown by the plant. While this effect is thought to be brought about by the introduction of reducing conditions, it is by no means an established fact and further investigation is necessary.

So far no satisfactory field control has been achieved by applying chemical reducing compounds as distinct from acidifying agents.

Data referring to the composition of the plant under normal and manganese deficient conditions are now being accumulated in this country. Table VI shows the effect of treatment on the manganese content of roots, laminae and petioles of mangolds.

Distribution of the disease

An examination of the voluminous literature dealing with manganese deficiency and its control suggests that the disease is widespread. The countries where it appears

Table VI. *Manganese content (p.p.m. of Mn) in dry matter*

	Mangolds		
	Roots	Laminae	Petioles
Control	18	20	16
Artificials	24	30	15
$MnSO_4$ + artificials	40	180	46
$MnSO_4$ alone	26	86	20

to be of importance occur both in the new and old worlds and include America, Australia, Japan, Holland, Scandinavia, Great Britain and many others. Just how important it is in our own country it is difficult to say, but there is no doubt that both peat, and mineral soils high in organic matter such as are found in reclaimed heath and moorland areas, are fairly extensive, particularly in certain localities. The area of fenland alone exceeds a million acres. The disease is associated with definite soil types, such as would be identified and mapped by the soil survey. This, among many other good reasons, points to the necessity for increasing the activity of soil surveys in this country and, until that is done, the actual area where manganese deficiency diseases may occur under natural or artificially induced conditions must remain a matter of speculation.

Practical implications: danger of over-liming

No soils, so far, have been discovered in this country in which manganese is absent. Certainly, some contain very low amounts (0·004%), but under suitable conditions these are adequate for the plants' needs. It has been shown that raising the reaction of a soil above a *p*H value of 6·5 is conducive to the occurrence of disease. This effect is not generally apparent in ordinary mineral soils but occurs in those with a high organic content. No definite statement as to the amount of organic matter which must be present can be made, since this figure will vary according to the amount of the mineral part of the soil. Black sands, on which the disease is present, may have an organic matter content as low as 6%. Peat soils, on the other hand, have an extremely variable organic matter content and may be as high as 90%. Certain natural conditions, such as the occurrence of an alkaline subsoil, lead to the appearance of the disease in crops and are largely outside the control of the farmer. The rate of application of lime, however, does come under the control of the farmer and it is essential that care be exercised on susceptible soils as to the amount applied.

As a working principle it is suggested that a *p*H of 6 is sufficiently high for the needs of practically all crops on any soil. If below this, it is evident that only such an amount of lime should be given as a dressing which will raise the *p*H to this figure, at any rate on susceptible soils. Not only must the quantity be controlled but the type of lime used and the method of application are points equally important. It is suggested that a ground form of lime (either carbonate or oxide) should be used, one-half applied before and one-half after ploughing, both applications being thoroughly worked into the soil. Only by some such method would it be possible to obtain really adequate mixing and to avoid the excessive alkalinity due to localized effects which may otherwise result. This problem is particularly urgent at the present time with the widespread increase in the use of lime being made under the stimulus of the Land Fertility Scheme. While it is particularly important that nothing should be said

which will in any way curtail the effort at present being made, a word of warning to the farmer to exercise care under certain circumstances is certainly necessary.

In the West Midlands, as indeed elsewhere, very considerable areas of black soils occur and on these care is being exercised in the amount recommended in all cases where fields are examined and samples taken by responsible persons.

In those areas where susceptible soils are prevalent, doubtless, as in other areas, many farmers apply lime without recourse to sampling. These are the difficult cases, since the farmers by their own zeal may ultimately injure their crops.

Too much caution cannot be exercised by all those who act in advisory capacities, particularly with respect to recommendations of lime on soils wherein manganese deficiency may develop.

(The paper was illustrated by lantern slides.)

II. THE EFFECT OF BORON ON THE GROWTH AND QUALITY OF SUGAR BEET

By A. W. Greenhill, Ph.D., M.Sc., F.I.C., A.R.C.S.

Director, Boron Agricultural Bureau, London, S.W. 1

It is now recognized that the presence of a small amount of boron is essential for the healthy growth of the beet plant. This was first discovered, about eight years ago, by the German scientist Brandenburg, who grew beets in water cultures. In the absence of boron the plants remained backward in growth and developed certain disease symptoms, the characteristic feature of which was the withering and death of the heart leaves. The plants to which boron was supplied were completely healthy. Brandenburg further showed that a small supply of the element is required by the beet plant at all periods of its growth. His findings have been confirmed in this country by Rowe at Rothamsted, and by workers in other countries. Rowe found that the apical meristem of the shoot, the youngest leaves and the newly developed cambiums of the beet are the parts most sensitive to boron deficiency, and the first to degenerate. Hypertrophy of the cambial cells and of the adjacent parenchyma cells, together with necrosis and disintegration of the phloem, characterize the later stages of deficiency. Evidence was obtained that the first indication of boron deficiency is plugging of the sieve-tubes.

Brandenburg extended his investigations to the field. A disease of sugar beet, in which the heart leaves withered and died, had been known commonly in practice for many years, though its cause had remained obscure. He treated some of the soils upon which the disease occurred with a small quantity of borax or boric acid, and found that it controlled the disorder. His discovery led rapidly to the conduct of many similar and equally successful experiments in the chief European countries, including the British Isles, and in the sugar beet growing districts of the United States.

Symptoms of Heart Rot disease

The disease is known generally as Heart (or Crown) Rot. It becomes apparent usually in late July or in August or September, following a period of dry weather. The first visible symptoms are seen always in the *youngest or heart* leaves, which cease to

grow and become curled. Dark brown patches appear on their stalks, chiefly on the inner sides, and the stalks themselves are found to be abnormally brittle. Within a short time, the heart leaves wilt and turn yellow and soon blacken and die: the main growing point is killed. The older, outer leaves may become affected in the same way later, especially if the dry weather continues. In severe cases all the leaves may die. Sometimes, and more particularly with the onset of rains, groups of young leaves develop from secondary growing points, giving the plant a characteristic short, green top and often tending to mask the original symptoms. These leaves may also become affected later.

The symptoms shown by the leaves are followed generally by a characteristic discoloration and rot in the root, to which the name Dry Rot is usually given. The crown becomes brownish or black in colour. Depressed spots develop and penetrate into the flesh in the form of a Dry Rot. These spots gradually increase in size and in badly affected plants may bring about the destruction of a large part of the root. In some cases, also, internal brown streaks spreading down the root may be seen on cutting it open longitudinally.

Effect of disease on yield and sugar content

Not only does Heart Rot reduce the yield per acre; the disease is also accompanied by great changes in the metabolism of the plant, affected beets containing a lower percentage of sugar than healthy ones. Thus there is a double loss, and the reduced monetary return from an affected crop is often very considerable. In a severe attack in Norfolk investigated by Hanley & Mann in 1935, the yield from a 16-acre field, on the factory returns, was only 2·3 tons of washed beet per acre. The average sugar content was 14·3%, and a trial lifting of the more severely affected beets showed a value of only 8·1%. The danger of such roots being included in the sample for analysis at the factory will be appreciated. In another case in Norfolk, which the same workers investigated, the presence of the disease reduced the yield of sugar per acre by over 30%. An average reduction of 38% in the yield of sugar was reported for the same year by the Department of Agriculture, for twenty centres on which the disease occurred in the Irish Free State. The serious nature of such losses needs no emphasis.

Influence of soil and weather conditions on Heart Rot

Heart Rot occurs to a varying extent in practically all beet-growing countries, including the British Isles. The annual loss to farmers in Poland through the disease, for instance, has been given as about the equivalent of £300,000 sterling. In Germany, it is estimated that somewhere between 150,000 and 200,000 acres of sugar beet were treated with boron in each of the past two seasons. Whilst the disease occurs on a variety of soils, it is most prevalent on the lighter types. It may, however, appear on the heavier soils, as instanced by the attack reported in 1935 at Rothamsted.

The disease is most frequently associated with alkaline conditions in the soil. Attacks on acid soils have, however, been recorded. The effect of heavy applications of lime in inducing the appearance of the disease has now become widely recognized, and several official warnings have lately been given of this possible danger from over-liming.

The soil moisture content appears also to play an important part in determining the incidence of Heart Rot. The disease is associated mainly with a low soil moisture

content, and so with dry summers. The conditions most conducive to its occurrence appear to be a wet spring, in which growth is rapid and unchecked, followed by hot, dry conditions in July and August. Soils bearing an affected crop in a dry year may, in a wet season, produce a crop which is relatively or completely free of Heart Rot. The disease often occurs in patches in a field. In the case of sloping or undulating land, it will usually be more noticeable on the higher, drier parts. Generally speaking, the largest plants in a field are likely to be the first to be affected.

Heart Rot due to boron deficiency

It is of interest to record the earlier views on the cause of Heart Rot, and the modification of these following the investigations of Brandenburg and others in recent years. The early investigators noticed that the fungus *Phoma Betae* was generally present in the diseased tissues of affected beets. They concluded that this fungus was the direct cause of the disease. It became recognized, however, that *Phoma Betae* was a weak parasite, able to attack only beets whose powers of resistance had been lowered in some way. We now know that where Heart Rot is accompanied by *Phoma Betae* attack, the latter is of a secondary nature. Subsequently, the view came to be held that Heart Rot was due to physiological disturbances, associated usually with alkaline soil conditions.

From the evidence available, it is now generally accepted that Heart Rot is due to a deficiency of boron. This deficiency may occasionally be a primary one. In the majority of cases, however, it is probably induced by alkaline soil conditions rendering the boron naturally present in the soil unavailable to the plant. This effect is most marked under conditions of low soil moisture content, and on soils of low water-retaining capacity. Whether it is due to direct chemical reaction, or to biological factors, or to both, is still uncertain.

Several observations made recently further confirming the present view are worthy of mention. Brenchley & Watson, following the attack of Heart Rot at Rothamsted in 1935, collected some diseased roots from the field, and transplanted them into sand cultures. Boron was added to some of the cultures and omitted from others. Except where the disease had already done irremediable damage before transplanting, the boron-treated plants all produced healthy shoots. The plants which received no boron, on the other hand, produced shoots showing the characteristic signs of deficiency. Cook, at the Michigan Agricultural Experiment Station, U.S.A., has confirmed the findings of Brenchley & Watson, by cutting diseased roots into halves and growing the halves in culture solutions containing boron and without boron respectively.

The view that Heart Rot is due to a deficiency of boron was criticized earlier on the ground that healthy roots and those suffering from Heart Rot contain similar amounts of boron. Recently, Brandenburg has shown that although this is so, large differences exist between the boron contents of the leaves of healthy and diseased plants. He obtained the following figures in 1935 and 1936:

	mg. H_3BO_3 kg. dry matter	
	1935	1936
Leaves of diseased plants	93	72
Leaves of healthy plants from the same field	202	161
Leaves of healthy plants from fields free from Heart Rot	220	236

Brandenburg states: "The boron taken up by the beet is for the most part fixed in the leaves, and can be further mobilized only with great difficulty....The leaves formed during a deficiency period have an extremely low boron content, whilst the outer leaves still show the normal amount."

Boron treatment of Heart Rot

It would be expected that any factor which has the effect of increasing the amount of available boron in the soil would tend to reduce the incidence of Heart Rot. We see this in the influence of wet summers when the soil moisture content is maintained at a relatively high level. Also, it has been shown that the addition to the soil of materials which reduce soil alkalinity, lessens the incidence of the disease.

In general practice, however, the best method of treatment has been found to be the application to the soil of from 15 to 20 lb. of borax per acre. This should be applied preferably when preparing the land for sowing, but it can be given during the early growing period, or even later when the first symptoms of disease appear. This late application, however, is usually less effective than is an earlier one. To apply satisfactorily the small quantity required, the borax may be incorporated in the ordinary fertilizer mixture, or it may be given as a separate application after admixture with a spreader to provide sufficient bulk for even distribution. In view of the known toxic effects of excess amounts of boron, uniform mixing of the borax with the other materials, and even application over the land, are important. Further details concerning the application of borax to soils, and its admixture with other fertilizers, are given in various publications on the subject.

In practically all of the many experiments which have been conducted in recent years in a number of countries, boron treatment of the soil has completely, or almost completely, eliminated the symptoms of Heart Rot. At the same time there has been a considerable increase in the yields of roots and tops, and in the percentage sugar content of the roots. Some typical results obtained in experiments conducted in this country and in the Irish Free State are shown in Tables I and II respectively.

Table I. *Effect of borax on sugar beet in Norfolk*, 1935*

Borax applied lb./acre	Beets showing Heart Rot %	Yield of washed beet tons/acre	Sugar content %	Yield of sugar		Weight of tops tons/acre
				cwt./acre	% increase	
0	51·2	5·7	14·4	16·5	—	2·3
4	42·5	6·2	14·9	18·4	12	3·4
14	6·1	7·1	15·9	22·5	36	4·1
28	5·9	7·5	16·2	24·1	47	4·3

* Hanley, F. & Mann, J. C. (1936). The control of Heart Rot in sugar beet. *J. Minist. Agric., Lond.*, **43**, 15–23.

The application of borax to beet crops at rates above 20 lb./acre is not usually necessary, and, in view of the toxic effects of excess amounts, is not generally to be recommended. It is of interest to note, however, that at the Norfolk Agricultural Station, in the seasons of 1936 and 1937, applications up to 1 cwt./acre produced no apparent harmful effects on sugar beet or the following crop. The number of such tests which have been carried out is, however, small, and there is evidence that similar results would not obtain on all types of soils.

Table II. *Effect of borax on sugar beet in Irish Free State*, 1935*
(Average returns from 20 centres)

Borax applied lb./acre	Incidence of Heart Rot	Yield of roots (net factory wt.) tons/acre	Sugar content %	Yield of sugar	
				cwt./acre	% increase
0	Considerable	8·2	16·6	26·9	—
14	Less extent	11·1	18·1	40·0	49
21	Very small	11·6	18·3	42·3	57
28	Practically free	11·9	18·3	43·6	62

* Crown Rot in sugar beet (1936). *J. Irish Free State Dept. Agric.* **34**, 131–2 A.

Unfortunately, there is no ready means at present of examining a soil chemically in the laboratory and saying whether or not it is deficient in boron. If its boron content is determined, the figure can have only a limited value, since a number of factors appear to influence the availability of the element to the plant. The only sure means at present of diagnosing boron deficiency in the soil is the observance of deficiency symptoms in the plant.

In view of the very striking results obtained in the control of Heart Rot by boron treatment, experiments have also been carried out on soils from which the disease was absent. The application of borax under these conditions has been found usually to be without effect on either the yield or the percentage sugar content of the beets. In some cases, a small increase in yield has, however, been obtained.

In the absence of Heart Rot, it is generally assumed that the natural boron content of the soil is adequate for the needs of the beet crop. This is no doubt mostly true. The possible future development of a condition of boron deficiency in more of our sugar beet soils should, however, be kept in mind. The appearance of Heart Rot is almost certainly indicative of an advanced stage of deficiency, and there are probably many soils at the present time in which less acute conditions of deficiency exist. Concerning these we at present know very little. In the past, the boron reserves of the soil have no doubt been maintained in some measure by the use of farmyard manure and some of the older chemical fertilizers, which contain appreciable traces of the element. With the present decreasing use of farmyard manure, and the reduced minor element content of some of the modern chemical fertilizers, those former sources of supply are diminishing.

The question is often asked: How long does a dressing of boron remain effective? From the evidence so far obtained, it appears that the normal dressing of 20 lb. borax/acre may be expected to remain effective for two or three years at least.

The fear has sometimes been expressed that the application of boron to beet soils may in time result in the accumulation of the element in the soil to an extent which may be harmful to other crops less tolerant of boron. The results of leaching experiments carried out recently in Germany showed, however, that a large proportion of the added boron not taken up by the crop is likely to be leached out of the soil by rains, and that no harmful accumulation is likely to occur.

III. SUGAR BEET PESTS

By F. R. Petherbridge, M.A.
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The Black Bean aphis: Aphis (Doralis) fabae *Scop.*

This aphis has previously been known in this country as *Aphis rumicis* L. Continental workers, however, use the name *Aphis* (*Doralis*) *rumicis* L. only for the non-migratory aphis which lives on docks.

We have recently found a black aphis which oviposits on *Rumex* and undoubtedly conforms to the continental description of *Aphis rumicis* L. (D. Hille Ris Lambers, 1934, *Stylops*. **3**, 25). This aphis is quite different from the Black Bean aphis which has hitherto been referred to as *Aphis rumicis* L. but should now be called *Aphis fabae* Scop. We suggest therefore that the writers who have mentioned oviposition on *Rumex* are referring to *Aphis rumicis* L., a non-migratory species according to continental authorities, and not to the Black Bean aphis. It is not denied that the latter migrates to *Rumex* as well as to other summer hosts.

At present we have only the oviparous form of the true *Aphis rumicis* and know nothing of its biology other than that it curls the leaves of *Rumex* and oviposits on the dead leaves.

Differences between oviparous forms of *Aphis rumicis* and *A. fabae* are as follows:

(1) *A. rumicis* distinctly larger in length of body, cornicles, etc. Length 2·5 mm. *A. fabae* 1·7 mm.

(2) Skin of *A. rumicis* very strongly reticulated. *A. fabae* slightly.

(3) Hind tibiae of *A. rumicis* with few sensoria but otherwise normal. Hind tibiae of *A. fabae* with numerous sensoria and markedly broader or flattened.

Börner further splits *Doralis fabae* Scop. into *D. euonymi* F., *D. viburni* Scop., *D. philadelphi* Börner and *D. mordvilkoi* Börner, but Ris Lambers says: "With the exception of *D. viburni* Scop. which has apterous males, I should prefer to treat them as forms of *D. fabae* Scop. with small differences in biology."

The attack of this aphis on sugar beet was exceptionally severe in 1938 and caused more damage than previously experienced. We estimate the average loss of crop as being over one ton of sugar beet per acre.

Colonies were first found on *Euonymus europaeus* on 9 April and winged forms on 27 April. They were not found on broad beans in gardens until 21 May, but were noted on sugar beet on 23 May and on the ordinary beet crop on 27 May. Enormous colonies were formed on the sugar beet crops during June and early July but at the end of July they had practically disappeared. Predators, especially ladybird larvae, were exceptionally abundant and were responsible for the early disappearance of the aphides. We were unable to find them on the sugar beet crop after the middle of August, but one sugar beet plant (a bolter) in a field near Norwich was found smothered with aphides on 17 September. No other aphides were found in this field.

Return migrants were first found on *Euonymus europaeus* on 16 September. There were very few males and in the Cambridge district the number of eggs laid was very few.

Observations were made on the primary distribution of this aphis; except in one bean field it was not mainly confined to the headlands.

Experiments were carried out to test the value of the following insecticides in controlling the pest on sugar beet seed: (1) quassia extract; (2) quassia extract plus soft soap; (3) nicotine—soft soap; (4) a proprietary preparation containing pyrethrum, atomized; (5) paraffin, atomized; (6) nicotine dust.

Nicotine dust gave the best control and was considered to be a commercial proposition. One seed grower in Bedfordshire carried out our instructions involving topping badly infested plants and then dusting with 3% nicotine dust. His total expenses on a 10-acre field were £2. 4*s*. 5*d*. per acre. His yield was $16\frac{1}{3}$ cwt./acre, which was considered very satisfactory indeed for a drought year like 1938. 3% nicotine dust is effective on warm days. 4% nicotine dust gives better results on colder days.

Our observations on the treatment of the ordinary beet crop for this pest suggest that none of the methods used is an economic proposition. With the machines at present on the market it is difficult to reach the aphides with dust, and there is a great need for machines which will deliver dust in such a way that it will reach the lower surfaces of the leaves. Atomizing with a preparation containing pyrethrum was not satisfactory and occasionally caused scorching, and in one case it actually caused a heart rot.

Wireworms

The important damage caused by this pest occurs in April and May when the sugar beet is in the seedling stages. If a sufficient stand of beet can be obtained, then the crop is usually a satisfactory one because the loss caused later in the season is not serious.

In 1937 experiments were carried out in a Fen field ploughed out of old grass in 1936, and where the beet had followed a chicory crop spoilt by wireworm in 1936. Here, by drilling wheat between the rows of beet at the same time as the drilling of the beet, a satisfactory stand of beet was obtained with seed at the rate of 15 lb. and 20 lb./acre respectively. Even the rows with wheat on one side only gave a fairly good stand. The rest of the field was ploughed up and re-drilled.

The yield of the original sowing was 14 tons $3\frac{1}{2}$ cwt./acre, whereas that of the re-drilled portion was 11 tons $5\frac{3}{4}$ cwt./acre.

In 1938 a number of fields were sown with beet and intersown with wheat directly after ploughed-up grassland. The wireworm population of these fields varied from 2–10/sq. ft. In all cases a satisfactory stand of beet was obtained. In one field with a population of 20 wireworms/sq. ft. the experiment was interfered with by birds.

These experiments suggest that sugar beet may be satisfactory as the first crop after ploughed-up grassland provided the land is suitable for growing beet for other reasons than wireworm.

Beet eelworm (Heterodera schachtii *Schmidt*)

This pest, which attacks both chenopodiaceous and cruciferous crops as well as certain weeds such as dock (*Rumex obtusifolius*), knot weed (*Polygonum aviculare*) and hemp-nettle (*Galeopsis speciosa*), was first found in England in 1934 and has now been found in 96 different fields. Many of these are beet-sick.

The following table shows the distribution:

	No. of fields
Bedfordshire	1
Cambridgeshire	1
Isle of Ely	41
Norfolk	45
*Leicestershire	3
*Lincolnshire	2
†Dorset	3

* Reported by A. Roebuck. † Reported by W. E. H. Hodson.

American workers suggest that the sugar beet eelworm was introduced into America by means of cysts present in small clods of soil mixed with imported beet seed. We have found cysts containing embryonated eggs in the dirt from imported seed but none of these eggs was viable. We have, however, found viable cysts of the potato strain of *Heterodera schachtii* in dirt from English seed.

The following suggestions have been made to assist in checking this pest:

(1) In fields or smallholdings where beet-sickness has occurred or where the egg count is high, sugar beet, mangolds, beetroot, spinach, turnips and swedes, and if at all possible other cruciferous crops, should be omitted from the rotation *for at least five years*. Before these crops are grown again, the egg count of the soil should be determined and expert opinion obtained.

(2) Where beet eelworms are present and where the egg count is low, the crops mentioned in (1) should be omitted for three years.

(3) Where no beet eelworms are present the above crops should be omitted for two years.

As the eelworms are readily spread from field to field by means of carts, tractors and other farm implements, workers' boots or the legs of horses, sheep and cattle, it is desirable to take special precautions in infected areas.

In the case of smallholdings we suggest that special arrangements should be made to enable them to grow potatoes as a substitute for sugar beet.

We further suggest that certain areas embracing a number of infected fields and also fields not known to be infected, should be scheduled as "sugar beet eelworm infected areas". In these areas sugar beet, mangolds, beetroot, spinach, turnips, swedes and other cruciferous crops should be grown only once in three years or preferably once in four years.

Lantern slides were shown illustrating some of the points of interest of the above and other pests of the sugar beet.

IV. PESTS OF THE SUGAR BEET CROP IN THE MIDLANDS

By A. Roebuck, N.D.A.

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Curtis in his *Farm Insects* quotes Dickson's *Practical Agriculture* as saying that mangolds are not injured by insects or drought. He adds that such appeared to be the case until about 1844 when *Silpha* appeared in Ireland and France. His full list of pests is: (1) *Silpha opaca*, (2) *Cassida nebulosa*, (3) *Phyllotreta nemorum*, (4) *Atomaria*,

(5) *Pegomyia*. That was the position before 1850. The sugar beet crop, as distinct from mangolds, might be said to be a post war introduction. Have we reached normality, if such a thing exists, in the pests which attack it? Looking back over the period from 1922, in the Midlands, one's general impression is that there has been a change. In the first year or two of the crop rabbits and *Pegomyia* were the only pests noted. Nowadays *Pegomyia* has lapsed into insignificance and rabbits are accepted as necessary evils. To-day the general pests such as wireworms, slugs, leatherjackets, etc., when abundant, take toll of the crop, as of all other farm crops. In addition to these there are a few pests, such as *Atomaria* and flea beetles, which attack the crops very severely in certain seasons and then apparently vanish for a number of years. At the present time there hangs over the whole industry the fear of *Heterodera*, which has begun to attack the crops in recent years.

Altogether, we have recorded 24 species of pests doing damage in the Midlands on this crop. In this total wireworms, slugs, flea beetles, etc., are counted as single species only.

It is interesting to notice what the status of Curtis's five species of pests has been on the sugar beet crop:

1. *Silpha* spp. He cites *Silpha opaca* and possibly other species. This has not made any headway. We had records in 1924, 1929 and 1936, but in no case was appreciable damage done. In our case the species is not *S. opaca* but mostly *S. atrata*.

2. *Cassida nebulosa* has even made less progress as a pest. We had *Cassida vittata* and *C. nobilis* eating a few leaves on several crops in 1936, but no assessable damage was done.

3. *Phyllotreta nemorum*. The flea beetle recorded nowadays is *Chaetocnema concinna*. In the Midlands flea beetles caused much damage in 1930 and were serious pests in 1935. In both years probably more damage was done by *Phyllotreta* spp. (*P. undulata* and *P. nemorum*) than by *Chaetocnema*.

4. *Atomaria linearis* is still at times a most serious pest. We have had two bad years and two years of moderate attacks. It was a moderately severe pest in 1925, then bad in 1929, moderate again in 1931 and bad in 1935. Fortunately this pest and flea beetles have only been destructive in the same year once, namely in 1935.

5. *Pegomyia hyoscyami* var. *betae* remained with us more frequently but has not been a serious pest. It was most frequent in 1923, 1924, 1925, 1929 and was local in 1930, 1932 and 1937.

Now let us consider the other pests to-day. *Carabid* beetles have attacked odd crops in different parts of the Advisory Province, especially in the years 1924, 1931 and 1938. The species concerned are *Pterostichus vulgaris*, *Pt. madidus* and *Ophonus pubescens*. Miss Ormerod recorded *Pt. madidus* on mangolds in 1885 near Bishop's Stortford. The damage is characteristic. A hole is bitten from an exposed part of the plant, e.g. the top of a root or a petiole of a lower leaf. Why they should attack crops at all is difficult to say. In this respect they compare with the Silphid beetles. In all cases trapping them by folded sacks soon stops the trouble.

Of *Aphis fabae* it is interesting to read in Curtis that: "During the summer of 1847 the prodigious swarms of aphides (*A. fabae*) which suddenly covered the young shoots and undersides of the leaves of almost every plant, so that the surface was blackened by them, was unprecedented." The year 1938 was a similar season. The aphides appeared to be quite content on any herbaceous plant until the end of July, when they left

them abruptly. In spite of this they did not do much damage except on the southern boundary of the Advisory Province, where crops were often completely defoliated. Everywhere, however, they were a constant source of anxiety. *A. fabae* first appeared as a pest in 1929, then again in 1931 and 1937. There were some slight late attacks in 1930. Early attacks, which soon disappeared, occurred in 1933 and 1935.

Chafer larvae (*Melolontha*) have an interesting record in Nottinghamshire and Lincolnshire. They have appeared as pests at 4-year intervals, namely 1928, 1932 and 1936. In 1930 there were one or two attacks and in 1938 two fields were attacked, one in Nottinghamshire by 3-year-old larvae and one in Lincolnshire by 2-year-old larvae.

Plusia gamma has also an interesting record but is of less importance. To Curtis it was a turnip pest and to Miss Ormerod it was the beet moth. She instanced a great attack by these caterpillars in 1879. In this Advisory Province it appeared in fair numbers in 1933, especially in Lincolnshire. In 1936 the caterpillars appeared all over the Advisory Province on every crop, making large holes in the leaves. In Derbyshire, out of the sugar beet range, the swedes were attacked.

Of the general pests, *Cutworms* (*Agrotis* spp.) have been the most troublesome. They appeared in 1924, 1929, 1931, 1932 and 1935. They have been very puzzling the last two seasons. They were abundant in the fields during the autumn, but they did not attack the beets.

Millipedes are a source of anxiety. In some fields they persistently remain and destroy the germinating seeds, in others they nibble the young roots.

Leatherjackets and *slugs* have varied much in abundance from year to year. The former have attacked crops in four seasons and the latter in two seasons during the period under review.

Heterodera schachtii has appeared at five centres:

(*a*) It was first recorded in the Midlands in 1936. On the same field, in the Isle of Axholme, sugar beet, mangolds and swedes were all attacked. Sugar beet had only been grown on part of this field once before, namely in 1935; red beet had been very frequently grown.

(*b*) In 1936 a field of swedes near Barton-on-Humber was attacked by *Heterodera*. There is no record of mangolds or sugar beet ever having been grown in this field.

(*c*) In 1937, swedes in a garden near Lincoln were attacked. In neither of these two cases would the eelworm attack sugar beet.

(*d*) In 1938 the eelworm appeared at Loughborough, attacking mangolds and sugar beet. It was ultimately found on cabbages after a long search, but they did not seem to be affected by the parasite.

(*e*) During this winter the cysts were found in a field in Derbyshire near Burton-on-Trent, which had just grown a crop of sugar beet. They were very sickly and could not have fed on the beet but probably on the various *Brassica* crops which had been grown in previous years.

It would seem that there is every gradation in these cases between a wholly *Brassica* strain and a strain which attacks beet and *Brassica* spp. equally.

Another eelworm appeared in November 1936, namely *Anguillulina dipsaci*. This appears on the maturing beets and produces a characteristic powdery corky canker round the crown. It is widespread but the losses are not great because of the lateness of its appearance. This is probably the same strain which attacks potatoes at uncertain intervals.

V. SUGAR BEET DISEASES

By H. H. Stirrup, M.Sc.

Midland Agricultural College, Sutton Bonington, Loughborough

Instead of giving a set paper, I propose to show about 50 lantern slides illustrating various sugar beet diseases and to comment upon them.

Most of the troubles that affect sugar beet in the seedling stage are grouped together by farmers and others and regarded as a single disease, this being called Black Leg. Used in this way, the term really covers a number of troubles, due to different causes. The first few slides illustrate the symptoms of the following seedling troubles: (1) True Black Leg, (2) insect injury, (3) damage due to soil acidity and (4) wind damage.

The first essential for healthy seedling growth is a good seed-bed. Cloddy seed-beds result in weakly seedlings that are unable to withstand the attacks of various parasitic fungi; the result is the loss of many seedlings from various causes.

True Black Leg is caused by certain fungi attacking the hypocotyls of seedlings growing in unfavourable conditions. The most important of these fungi is the seed-borne *Phoma Betae*, but species of *Pythium* and *Rhizoctonia*, which are soil inhabitants, are also concerned. The following slides show the symptoms of varying degrees of attack by the fungi that cause Black Leg.

The chief adverse growing conditions which render seedlings liable to attack by these fungi are: (1) poor tilth in the seed-bed, (2) low soil temperature, (3) shortage of soil moisture, and (4) cold winds. Numerous experiments have shown that seed treatment using an organic mercury compound exercises a certain amount of control of Black Leg when the seedlings are growing under adverse conditions, and also increases the number of seedlings emerging through the soil. When, however, soil and weather conditions are favourable for germination and young seedling growth, little or no benefit accrues from seed treatment.

There are two distinct types of injury to seedlings associated with soil acidity. (Slides illustrating the symptoms of these troubles.) In the first type, the damage is confined to the radicle and young lateral roots, the hypocotyl remaining unaffected. The root system becomes tough and string-like and this type of acid soil injury has been provisionally named "Stringy root". The second type of injury occurs on the hypocotyl only; instead of increasing in size in the normal manner, the hypocotyl remains thin and tough and its surface becomes brown and scurfy. This type of injury is provisionally named "Stringy hypocotyl" and at certain stages it is almost indistinguishable from Black Leg. Various fungi, chiefly species of *Fusarium*, have been isolated from the roots and hypocotyls of seedlings with acid soil injury and it seems likely that these weakly parasitic fungi are able to attack the underground parts of the plants only when they are weakened by growing under adverse acid soil conditions.

Strong winds can cause extensive damage to sugar beet seedlings, particularly on light, sandy soils and also on certain peaty soils which, when dry, assume a texture like that of fine ashes. Soils of this type are easily "blown". The damage may be done to the hypocotyls, cotyledons or true leaves. (Slides.) When the hypocotyls are affected, they become thin and shrivelled, and this type of injury is thought to be one of the early stages of Strangle disease. (Slides showing various stages of this latter

trouble.) It is not caused by insects, as was formerly thought. It is true that insects are attracted to the strangled part of the hypocotyl by the sugary exudate just before, or just after, the top of the plant breaks off, but such insects are merely secondary. Although there is no definite proof yet, it is thought that Strangle disease originates in the seedling stage as some form of internal injury to the hypocotyl near soil level. The formation of the constriction round the hypocotyl in that region is the result of rapid growth and increase in girth above and below the injury, but not immediately underneath it. Subsequently, if weather conditions are favourable for the rapid growth of the plants, they become top heavy and break off at the strangled region. Observations point to the fact that (*a*) acid soil injury and (*b*) wind damage, particularly when the seedlings are growing in a soil with a firm, crusty surface, may be two of the primary causes of Strangle disease.

Downy Mildew, caused by *Peronospora Schachtii*, has become much commoner on sugar beet in the last ten years and this disease is capable of causing considerable damage to both root and seed crops. Every year there is a cycle of infection from the seed to the root crops and back again to the young seed plants. Experiments are being carried out to find a method of breaking this infective cycle by spraying young plants in the seed-beds to prevent, or reduce the amount of, initial infection. One of the next seven slides shows a final stage of Downy Mildew attack, the thickened and distorted leaves having become a brown, shrivelled and desiccated mass; at this stage, it is very difficult to distinguish Downy Mildew from Heart Rot, the boron deficiency disease.

Of the four virus diseases of sugar beet known to occur in different parts of the world, Mosaic is the only one that has been recorded with certainty as occurring in this country. Of the non-virus types of Yellows, Crackly Yellows is by far the most important. The coloured photographs (slides) show all stages in the development of this trouble from the primary yellowing of the older, outer leaves to the final stages when the moribund parts of the leaves become invaded by various weakly parasitic fungi (chiefly *Alternaria* spp.) resulting in the so-called Leaf Scorch condition. The next two coloured slides show a different final stage of Crackly Yellows, called Red Leaf. The exact cause of this complex disease known as Crackly Yellows is not yet known, but it seems highly probable that shortage of soil moisture and nitrogen starvation are two of the factors involved.

I propose now to illustrate Speckled Yellows (slides), the manganese deficiency disease, and Heart Rot, the boron deficiency trouble, but in view of the papers read this morning I shall not comment on these diseases.

Rust, caused by *Uromyces Betae*, is not a serious disease of sugar beet in this country. It occurs most frequently in coastal regions and is usually most severe on good, well-grown crops. The two coloured slides illustrating this disease are particularly interesting because they show the cluster cup stage of the fungus, a rarity on sugar beet in this country.

The coloured slide of *Cerocospora* Leaf Spot shows a mild attack of this disease. Although comparatively unimportant in this country, this Leaf Spot is a very serious disease in certain continental countries and regular spraying operations have to be carried out there to control it. A high temperature and high atmospheric humidity are the necessary conditions before this disease can assume epidemic proportions.

The most serious root disease of sugar beet is Violet Root Rot (*Helicobasidium purpureum*). The two coloured slides show the symptoms on (*a*) a root just pulled

out of the ground with the mass of soil adhering to it and (*b*) a root from which the clinging soil has been washed, together with a section of a diseased root to show the depth to which the rot has penetrated.

The next slides show the symptoms of various other root rots, including (1) the black, shiny rot caused by *Phoma Betae* on a root not affected with Heart Rot, the boron deficiency trouble; (2) Wet Rot, caused by *Phytophthora megasperma*, a disease that occurs only in heavy soils that are unduly wet; (3) Internal Zoned Rot, a disease of unknown origin in which the internal tissues of the root contain well-defined zones of necrotic tissue; it has not been possible to isolate any parasitic fungi or bacteria from the affected tissues and it is considered that this disease is a physiological one; (4) Eelworm Canker, caused by *Anguillulina dipsaci*, already referred to in a previous paper.

The next two slides show the symptoms of lightning injury on sugar beet roots, the first on comparatively young plants and the second on older ones. The internal cavities can be clearly seen, and in the older root the point of entrance of the charge is visible. When lightning strikes a field of beet, the affected patch is roughly circular in shape and rather small, rarely more than 10 yards in diameter.

Finally, the last coloured slide shows the effect of spray injury on a sugar beet plant. In the early stages, the symptoms are very similar to those of Heart Rot, the boron deficiency disease, but later a secondary rot usually develops at the injured crowns. This type of injury occurred for the first time last season in a crop that had been sprayed with pyrethrum extract against aphis attack. It is considered that the damage was not caused by the pyrethrum but by the unsuitable oil base of the spray.

REVIEWS

Wood Pulp. By Julius Grant. Pp. 209. Leiden, Holland: Chronica Botanica Co. (London: Wm Dawson and Sons.) 1938. Guilders 7.

This book is intended to cater for those interested in plant science, and for students and general readers, but how far it will succeed in doing this is somewhat uncertain.

The work is divided into 20 chapters commencing with a general introduction in which wood pulp is defined and its relationship to other fibrous materials discussed. Thereafter follows a brief historical sketch of paper and pulp manufacture. Chs. III and IV, which deal with cellulose and the wood fibre, and with the identification and evaluation of pulping woods, and which should, therefore, be of direct interest to workers in plant science, are by far the weakest chapters in the book. The section on the chemistry of cellulose is far from being well done, and a certain looseness of expression tends at times to confuse the reader. For instance, it is difficult to reconcile the statement on p. 24 that the term "cellulose" does not signify a definite chemical entity, with a subsequent section on the molecular structure of cellulose: the more especially since no indication is given that the structure in question was established for the alpha cellulose of the cotton seed hair and not for wood cellulose. The few but none the less important papers on the constitution of wood cellulose are not dealt with. Free use is made of the term "compound cellulose" which was of more significance a generation ago than it is to-day. Most authorities would agree that to apply this term to hemicelluloses and particularly to holocellulose is a complete misrepresentation of modern opinion. Botanists will hardly be favourably impressed by the Section on the structure and growth of trees. Having stated early in Ch. IV that it is seldom necessary to identify a particular wood before it is pulped, the author proceeds to deal with the subject in some detail. An unfortunate misuse of the terms "hardwood" and "softwood" on p. 36 leads to confusion throughout the book wherever they are mentioned; tyloses are described as distended vessels. After stating that specific gravity varies from one type of wood to another the author invites comparison between cork and hardwoods in this respect. Several obvious mistakes are made in describing the determination of lignin in wood. It is stated that xylan is present in the cell walls of most trees except conifers. These and other examples which could be cited serve to show that the author is by no means at home with the botany and chemistry of his starting material. In describing the purely technical side of his subject he is obviously in known territory and a tolerably readable account is given of the mechanical, sulphite, soda and sulphate processes, but even here his incursions into the chemistry connected with the processes are marked by inaccuracies and confusion of terms. Semi-chemical pulping is not discussed but bleaching is dealt with in some detail. The chapters dealing with the by-products of the chemical pulping processes are informative. Representative lists are given of the tests applied to the finished pulp. The three short concluding chapters on the uses of wood pulp are among the best in the book.

W. G. CAMPBELL.

Manual for the Determination of Seed-Borne Diseases. By L. C. Doyer. Pp. 59, plates 33. Wageningen: International Seed Testing Association. 1938. Fl. 5.

This work has been compiled by Dr Lucie C. Doyer, Mycologist of the State's Seed Testing Station, Wageningen, in collaboration with the "Committee for the determination of seed-borne diseases". The booklet is in three parts: (1) a general section containing a brief classification of the conditions and germination behaviour of

contaminated or diseased seed, and an outline of methods of investigation; (2) a special section in which are discussed infections by fungi and bacteria and infestations by insects and nematodes in seeds of cereals, grasses, peas, beans and clover, beet, flax, cabbage, celery and parsley, carrot, spinach, lettuce, onion, salsify, tomato, corn-salad, and trees; the section closes with a note on saprophytic fungi present on seeds; (3) a tabulation of seed-borne infections and infestations arranged in parallel columns under headings according to method of determination.

The plates contain line, half-tone or colour illustrations of diseased seeds and the organisms parasitic upon them, and are loose in annotated folders.

The work is not a complete manual of seed-borne diseases but it includes most of the more common troubles and it is a very useful publication of unusual and pleasant format.

WILLIAM B. BRIERLEY.

Fungi of India. Supplement I. By B. B. Mundkur. Pp. iii+54. Scientific Monograph No. 12, Imperial Council of Agricultural Research. Delhi: Manager of Publications. 1938. 2*s.* 3*d.*

Butler and Bisby's *Fungi of India* (1931) recorded 2351 species, exclusive of the Myxomycetes. The present work adds 79 Myxomycetes, and 433 Eumycetes distributed as follows: Phycomycetes 54, Ascomycetes 67, Ustilaginales 6, Uredinales 66, Autobasidiomycetes 62, Fungi Imperfecti 178. There is one new species, *Mycosphaerella Tinosporae*, with description and Latin diagnosis, and six new combinations. Interesting notes are appended to most of the new records. There is a useful bibliography of 134 citations, and an index of genera.

WILLIAM B. BRIERLEY.

The Vegetables of New York. Vol. I. By U. P. Hedrick. (Part I. Peas of New York. Pp. vi+132. Plates 24. 1928. Part II. Beans of New York. Pp. iii+110. Plates 39. 1931. Part III. Sweet Corn. Pp. iv+111. Plates 24. 1934. Part IV. The Cucurbits. Pp. iv+131. Plates 49. 1937.) Albany, N.Y.: J. B. Lyon Co.

The magnificent volumes on tree and small fruits published by the New York State Agricultural Experiment Station under the general authorship of Dr U. P. Hedrick have long been familiar landmarks in horticulture. In 1925 a similar work was planned to deal in three volumes with the vegetables of New York, and the first volume has now been completed. The second volume will deal with salad crops and pot herbs, and the third with cole and root crops. Although the title relates the work specifically to the State of New York, the contents are more catholic and almost inclusive of North American vegetables.

The four parts of vol. I have been issued separately at intervals of a few years but, in each, a more or less standard plan is followed: History of the plants; systematic botany; detailed description of varieties; bibliography and index. It is not easy to do justice to the almost epic scale upon which the work is planned, the high quality which is sustained throughout, the fullness of treatment, or the accuracy of detail. Owing to the number of different plants to be considered a discussion of their culture, or of their botany, much of which is in need of investigation, would have enlarged the volume beyond necessity, but on such aspects as are considered the work is encyclopaedic. The historical chapters are fascinating, containing a wealth of allusion to early scientific and literary sources. The main portion of the work is concerned, naturally, with descriptions of varieties the selection of which has been guided partly by their horticultural and economic status, partly by their importance in plant breeding, and

partly by their value in illustrating systematic relationships and evolutionary trends. The actual descriptions are excellent: original and based on living specimens, and giving tersely yet fully an idea of all the characters of the several varieties. The style of writing is of high quality, simple, clear and condensed, yet interesting, and the beautiful printing, in double column on the page, makes the text easy to read. Lastly there are the illustrations, which are a sheer joy: full-page colour photographs, splendidly reproduced, and something almost unique even in horticultural literature.

In part I collaborating authors were F. H. Hall, L. R. Hawthorn and Alwin Berger; and in parts II–IV, W. T. Tapley, W. D. Enzie and G. P. van Eseltine.

WILLIAM B. BRIERLEY.

Cotton: History, Species, Varieties, Morphology, Breeding, Culture, Diseases, Marketing, and Uses. By H. B. Brown. 2nd ed. Pp. xiii + 592. London: McGraw-Hill Publishing Co., Ltd. 1938. 30*s.* 0*d.*

The first edition of this book received notice in the *Annals*, 1927, **14**, 564. In the new edition, which is larger by 75 pages, the general plan and chapter headings remain unchanged but, in every chapter, there have been considerable emendation, rewriting and addition of new paragraphs to bring the work up to date. Many of the tables have been amplified, the statistical tables usually include 1935 or 1936 data, and more recent references have been added to the bibliographies. Several new text-figures have been inserted and some of the earlier figures have been changed to illustrate later developments.

In spite of these alterations the book remains almost purely American in outlook; e.g. of the additional references in the new edition 80% are to U.S.A. publications. So far as it goes the book is a useful compilation but it might easily have been converted into a standard work.

WILLIAM B. BRIERLEY.

The Longevity of Plants. By H. Molisch. Translated into English by E. H. Fulling. Pp. 226. New York: Published by the Translator, Science Press Printing Co., Pa., U.S.A. 1938. $3.

Molisch's *Die Lebensdauer der Pflanze* (1929) is still the only book on this intriguing subject. The author discusses the longevity of unicellular and multicellular plants, longevity in relation to systematic relationships, organs and tissues, means of prolonging the life of plants, rejuvenescence, apparent death, and old age, death, and the alleged perpetual life of trees.

Although following closely the meaning of the German text, the translation is free and readable. Some of the tables have been rearranged, and some of the original illustrations omitted or replaced by excellent photographs by the U.S. Forest Service. The references, which in the German text were given as footnotes and often cited incompletely, are cited in full and relegated to a bibliography. Publications since 1928 are collated and classified in a second bibliography, and the names of the authors included in the author index. The subject index has been recast.

The writing of a new edition would have involved extensive reconsideration in view of more recent studies on vernalization, photoperiodism, plant and seed dormancy and the breaking of the dormant period, plant hormones, polyploidy, sex reversal, etc., and it is a great pity that this could not have been done since an up-to-date treatise is very desirable. Much of Molisch's work, however, remains valuable, and this excellent translation will serve to focus attention on an interesting and important problem.

WILLIAM B. BRIERLEY.

Plant Physiology. By N. A. Maximov. Edited by R. B. Harvey and A. E. Murneek. Second English Edition, translated and revised from the Fifth Russian Edition. Translated from the Russian by Dr Irene V. Krassovsky. London: McGraw-Hill Publishing Co., Ltd. 1938. 25*s.* 0*d.*

In preparing this new translation of Maximov's *Text-book of Plant Physiology* the editors have altered the title in order to avoid confusion with the former edition. It would have been even better if they had given it a title which would have indicated its very special character. The author himself in his preface to the Russian edition explains that his objective was to present "to the students of our universities and agricultural colleges and to our future technical agriculturists clear and exact information concerning the life and vital functions of green plants". The whole tone of the book is towards what has been termed "agrophysiology". It marks an entirely new departure in works on plant physiology; the plant is no longer considered as an organism to be analysed according to its separate functions but as a unit "in which all the parts and all the processes taking place in them are closely inter-related". It is the first time that what may be called the dynamic viewpoint, in contrast to the static outlook of the classical, mechanistic physiologists, has directed the writing of a text-book. The whole conception and arrangement of the volume is different from the usual standardized form.

The approach is first to the physico-chemical foundations of the plant and its mechanisms of metabolism. The discussion then passes, surprisingly though logically, to the earliest functions of the germinating seed, respiration and growth. The synthetic and absorptive processes are next reviewed, followed by chapters on the water relations of plants and the translocation of substances through the tissues. Ch. x is a masterly discussion of the resistance of plants to unfavourable environmental conditions, a subject on which the author is the primary authority. The remaining chapters are concerned with growth-correlations, the physiology of development, flowering and fruiting, and seasonal phenomena in the life of plants. In these chapters all the modern applications of physiology to agriculture and horticulture, including the hormone theory, vernalization, photoperiodism, fruit-ripening and so on, are reviewed more adequately than has ever before been done in such a book. Throughout, the outlook is on the plant as a dynamic organism, not a static thing to be analysed into its component parts and processes but a correlated whole whose parts and functions are mutually interdependent.

The editors have carried out their task in complete sympathy with the author, while at the same time introducing their own viewpoints. The book is not merely a translation from the Russian but has been widely modified to make it of more widespread interest. New illustrations and data have been used and references have been reselected from publications in English.

It would mean a revolution in our botanical schools if this could be taken as the standard work on physiology, but the gain would be immense. The new outlook is gaining ground in all the schools of applied biology; if it can invade the strongholds of the departments of pure science the mechanistic foundations will begin to crumble at last.

R. H. Stoughton.

Science in Agriculture. By J. W. Paterson. Pp. viii + 288. 165 illustrations. London: Longmans, Green and Co., Ltd. 1938. 6*s.* 6*d.* net.

This book does not attempt to teach farming, but deals with fundamental principles in a way likely to provide a healthy incentive to consider problems from the more practical aspect. It will be particularly helpful in schools where science with a rural bias is incorporated in the syllabus. To teachers who have to deal with this

subject, and more particularly to those who are town bred and have not previously studied agriculture, the book will make a strong appeal. It should give them that confidence in their subject which is so essential to success in imparting knowledge to others.

The author suggests that there is little reason why pupils in secondary schools should not receive their mental training from the study of Agricultural Science instead of the more orthodox chemistry, physics or biology, and his book goes far to make this possible. Its arrangement into numerous chapters, each dealing concisely with a clearly defined subject, makes it a handy book of reference to the farm pupil who wishes readily to gain information on a specific point. Some of the material may be a little too compressed, and a better balance might have obtained between the space devoted to crops as opposed to stock. One notable omission is that no reference is made to poultry, which not only make ideal subjects for practical school instruction purposes, but must now be considered as an important class of farm livestock. The book is written in a clear attractive style, and is well illustrated by up-to-date photographs and excellent sketches. It is of a handy size, and the printing is admirable, good use being made of heavy type and italics.

D. J. G. BLACK.

Report on Agricultural Research in Great Britain: a Survey of its Scope, Administrative Structure and Finance, and of the Methods of making its Results known to Farmers, with Proposals for Future Development. Pp. vi+146. London: P.E.P. 1938. 8*s*. 6*d*. net.

P.E.P. (Political and Economic Planning) is an independent non-party group of trained enquirers recruited from various professions, who devote part of their spare time to fact-finding and to suggesting principles and possible advances over a wide range of social and economic activities. Many of us are familiar with its fortnightly broadsheet "Planning", but from time to time P.E.P. issues full-scale Reports which are of permanent and constructive value.

The agricultural research organization in this country, born under 30 years ago, has developed so rapidly and branched out in so many different directions that, unless one is prepared to wade through numerous Government publications, it is not easy to form a mental picture of its structure and relations. This P.E.P. Report gives exactly such a picture, together with a critical appraisal. It is not concerned with the actual content of agricultural research but with its quality and quantity, the adequacy of the provision made for it, the efficiency and economy of its administration, and to what extent the present machinery for getting the results of research across to the farmer is successful.

The volume opens with a brief summary of the aims of the Report and of its findings and closes with six appendices listing institutions for agricultural education and research, and data relative to their expenditure and staffing, and a final appendix briefly outlining fishery and forestry research in Great Britain. The factual text of the Report is arranged in seven chapters of which the first is introductory. Ch. II surveys the structure of agricultural research in Great Britain and the relations of the multiplicity of governing and directing bodies, research and advisory organizations, and information bureaux. Ch. III describes briefly the finance of agricultural research and the various sources of money. Ch. IV outlines the personnel of agricultural research—numbers and subject distribution, grades and salaries, recruitment, etc. Ch. V gives an account of the ways in which scientific knowledge about agriculture is disseminated, and pointedly criticizes some of the rather glaring defects. Ch. VI contains a brief summary, based essentially on the last biennial Report of the A.R.C. (Agricultural Research Council), of what is actually being done in agricultural research —in short, whether an annual expenditure of some £700,000 of public money is being justified. Ch. VII is a brief statement, for purposes of comparison, of the general

organization and position of agricultural research in Northern Ireland, the U.S.A., and Denmark. It would have added enormously to the interest of this comparison if the U.S.S.R. could have been included. Each one of these seven chapters could with advantage have been made much fuller, although then, perhaps, the main lines of the picture might have been obscured by detail; as it is, the essentials of the position together with the defects in structure and relations stand out clearly.

The final chapter, "Criticisms and Conclusions", is the one which will be read with the greatest interest, although a certain amount of its fire is stolen by the summary with which the volume opens. It presents a number of reasoned criticisms and puts forward proposals for remedying certain defects or for changing certain trends in policy and practice. The main suggestions may be epitomized as follows: (*a*) that the Development Commission be eliminated and that the A.R.C. be transformed into an executive as well as a consultative body in matters affecting its own sphere of research policy, and perhaps in the dissemination of the results of research; (*b*) that the basic research institutes be augmented and that a parallel series of institutes on a husbandry and product basis be inaugurated; (*c*) that Research Directors be appointed to coordinate agricultural research; (*d*) that the Provincial Advisory Service be extended to include husbandry advisers; (*e*) that the County Advisory Service be reorganized; (*f*) that the Imperial Bureaux be extended and that a series of bureaux organized on a husbandry basis be inaugurated; (*g*) that the finance of agricultural research be reorganized, and an Agricultural Research Fund with contributions from producers and distributors be set up under the control of the A.R.C.; (*h*) that salary scales be raised; (*i*) that more technical assistance be available in research work; (*j*) that there be greater uniformity in the appointment of research officers; (*k*) that a Central Extension Service be established under the control of the A.R.C.

Now the above proposals, which are elaborated in the Report, cover a very wide field and penetrate deeply into all regions of the present structure and relationships of the research and advisory organization. Some of them are so obviously desirable that their implementing should only be a matter of the necessary action. Others, however, demand the keenest scrutiny for their implications are so far-reaching that in practice they would amount to a revolution. Whatever reservations one may have about particular proposals, however, there can be no doubt that in this Report P.E.P. has put forward a programme of constructive change which is a definite challenge to the present situation, and which demands the most serious consideration by the Government and by all interested in agriculture.

Of course, every reader of this Report will find additional or alternative points of criticism in the present conditions and wish to make further suggestions for change or reconstruction. For example, one of the main results of the P.E.P. proposals would be the concentration of much greater responsibility and power in the hands of the A.R.C. Now the Report takes the A.R.C. very much for granted, merely suggesting that it "might with advantage include a greater proportion of agricultural economists and practical farmers". But the present basis of constitution of the A.R.C. may give one seriously to doubt whether any Council so founded is competent to carry out efficiently even its present responsibilities and whether, perhaps, such a basis of constitution may not be fundamentally wrong.

Further, the rapidity and revolutionary nature of change in agricultural science and practice to-day, not merely in small things but in fundamental viewpoints, aims, methods and contacts, demands not only the mellowed wisdom of elder statesmen but also, and far more imperatively, the mental elasticity, the enthusiasm, and the intellectual vigour of youth. Whilst everyone is glad to render homage to the eminence of the present scientific members of the A.R.C. few can escape the conviction that their average age is, perhaps, 20 years too high.

Again, so far as research is concerned, the P.E.P. Report does not question the existing system of basic institutes but simply asks for more. In a Presidential Address to the Association [*Ann. appl. Biol.* (1934), **21**, 351], I criticized certain aspects of this scheme and, as yet, I see no reason to change my views. It may be protested that the existing scheme is a going concern and that, therefore, one cannot change it; but that is exactly the basis of criticism, for rigidity and crystallization into vested interests are anathema in research. Fluidity of monies, staffs, appointments, working facilities,

even buildings and experimental grounds, seems to me to be the very essence of a fruitful and progressive research organization. A living organization is one that carries within itself as an inherent and vital component the catalysts of its own change. And so one could continue until one had written a review longer than the Report.

The Report itself so intimately and immediately concerns nearly all the Members of the Association that it should receive the widest and most critical consideration by them, and it could profitably form the basis of a general discussion at one of our meetings. It is certainly one of the most important publications in its particular field that have appeared for many years, and no worker in any way connected with agricultural education and research can afford to neglect it.

WILLIAM B. BRIERLEY.

An Introduction to Botany. By A. W. Haupt. Pp. xii + 396. London: McGraw-Hill Publishing Co., Ltd. 1938. 18*s.* 0*d.*

Still another elementary botany textbook, of American origin and of about Intermediate standard, but distinguished from most of its fellows by its wider range and more balanced treatment, its higher quality, its original and beautiful illustrations, and alas! its higher price.

WILLIAM B. BRIERLEY.

Form and Causality in Early Development. By A. M. Dalcq. Pp. 197. 64 figs. Cambridge: at the University Press. 1938. 12*s.* 6*d.*

In few other regions of biology has such fundamental advance been made in recent years as in animal embryology, and the new order of ideas introduced and methodically developed by Spemann and his school has had revolutionary value. The principles originally introduced for the Amphibians have been applied with remarkable success by experimental embryologists to almost the whole range of Chordate germs, and to the apparently different embryonic forms encountered in Invertebrates, and embryonic regulation may now be considered a process of general occurrence. Thanks to the reciprocal illumination of recent descriptive and experimental results, the causality of animal form, which seemed beyond the goal of logical explanation, can now be given a satisfactory interpretation. The author has played no mean part in this achievement and, in this book, presents a thoughtful consideration of the data selected as being most significant at the present time for a general comprehension of the ontogenetic problem. Botanists can only wish that they had one tithe as much understanding of the similar problem in plants.

The volume is the first of a new series of "Cambridge Biological Studies" and it sets a high standard.

WILLIAM B. BRIERLEY.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1938

The Officers and Council of the Association were as follows:

President: C. T. Gimingham, B.Sc., F.I.C.

Vice-Presidents: H. Martin, D.Sc., H. Wormald, D.Sc.

Hon. Treasurer: J. Henderson Smith, M.B., Ch.B.

Hon. Secretaries: General and Botanical: W. P. K. Findlay, M.Sc.; Zoological: G. Fox-Wilson.

Hon. Editors of *Annals of Applied Biology:* General and Botanical: Prof. W. B. Brierley, D.Sc.; Zoological: C. T. Gimingham, B.Sc., F.I.C.

Council: H. F. Barnes, M.A., Ph.D., H. A. Dade, A.R.C.S., W. J. Dowson, M.A., D.Sc., T. Goodey, D.Sc., H. Martin, D.Sc., H. W. Miles, D.Sc., Ph.D., H. C. F. Newton, Ph.D., W. C. Moore, M.A., A. Roebuck, N.D.A., E. R. Speyer, M.A., H. G. Thornton, D.Sc., H. Wormald, D.Sc.

The Association has met on six occasions during the year, including two visits. The Annual Summer Meeting was held on 8 July at the Forest Products Research Laboratory, Princes Risborough, and the afternoon visit on 5 November to the Studios of the Gaumont British Instructional Films Co. at Shepherd's Bush, London: to both these institutions the Association is indebted for their kind hospitality.

At ordinary meetings the attendance as recorded in the signature book was, on the average, 39 Members and 18 visitors, an increase on the previous year: 28 Members attended the Summer Meeting, and about 50 the very popular visit to the film studios.

Twenty-one new Members were elected during the year and four Members resigned. The Council wishes to record with regret the death of Prof. Murphy who had been a Member since 1920. The Association now numbers 330 Members including 12 Honorary Members: of the ordinary Members as far as is known, 268 are resident in the British Isles and 50 in the Empire or in foreign countries.

The following papers and discussions were brought before the Association during 1938:

11 February. Presidential address by Dr J. Henderson Smith, *Some Recent Developments in Virus Research.*

18 March. *Discussion on the Use of Chemical Weedkillers.*

M. A. H. Tincker, M.A., D.Sc.: Weedkillers in Relation to Horticulture.

R. H. Macdowall, Dipl. R.T.C., A.M.I.Chem.E.: Some Factors influencing the Agricultural Use of Chemical Weedkillers.

G. E. Blackman, M.A.: The relative Toxicity of Chemical Weedkillers.

R. B. Dawson, M.Sc., F.L.S.: The Eradication of Weeds in Lawns.

O. Owen, M.Sc., Ph.D., A.I.C.: Chlorate Weedkillers.

7 October. *Discussion on Freshwater Biology.*

E. B. Worthington, M.A., Ph.D.: Introduction: Freshwater Biology and its Applications.

H. C. Mortimer, B.Sc., D.Phil.: Chemical Aspects of Organic Production in Freshwater.

A. C. Gardner, M.A.: Some Problems of Waterworks Biology.

Miss M. Rosenberg, D.Phil.: Algal Physiology and Organic Production.

9 December. *Observation on Apple Canker.*

R. G. Munson, B.Sc.: The Discharge and Germination of Ascospores of *Nectria galligena*.

R. W. Marsh, M.A.: The Incidence and Control of Canker Infections in Apple Shoots.

S. O. Garrett, M.A.: Recent Advances in Biological Control of Soil-borne Fungal Diseases.

The Association during the past year has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology and in the Metallurgical Lecture Theatre of the Royal School of Mines, and the Council wishes to take this opportunity of expressing, on behalf of the Association, its grateful thanks to the College authorities for the continuation of their valued hospitality.

The Hon. Editors of the *Annals of Applied Biology* report that in 1938 the volume comprised pp. xii + 891 and 37 plates, as against pp. xiv + 940 and 51 plates for 1937, this slight reduction being in accordance with a ruling of Council. Including papers published as "Proceedings", vol. **25** contained 61 papers and 41 reviews, as against 67 papers and 44 reviews for vol. **24**. Of the papers in the 1938 volume, 48 were by Members of the Association and 13 by non-members. The papers may be roughly classified as follows: General applied botany 15; Mycology and fungal diseases 17; Virus and virus diseases 6; General applied zoology 2; Entomology and insect pests 12; Plant protection 8; Helminthology and nematode diseases 1.

The several parts of the *Annals of Applied Biology* were published on the following dates: Part 1, 18 February; Part 2, 27 May; Part 3, 15 August; Part 4, 15 November.

W. P. K. FINDLAY
G. FOX-WILSON } *Hon. Secretaries*

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1938

During the year ending 31 December 1938 subscriptions and entrance fees, including arrears paid in, amounted to £324. 13*s.* 0*d.*, a decline of £13 as compared with last year. Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £763. 3*s.* 0*d.*, a decline of £46 due entirely to a falling-off in the sale of reprints, which is always a fluctuating item. The size of the *Annals* amounted to 891 pages as against 940 in 1937, and the cost of printing amounted to £1255 as against £1330 in the preceding year. In the whole year there has been an excess of expenditure over income of £90. 8*s.* 0*d.*

The Association has an excess of assets over liabilities of £762. 10*s.* 7*d.* In accordance with the policy of the Council we have again reduced the total of our assets by printing a volume larger than our income covers. We shall not, however, be able prudently to deplete our reserves in this way for more than two or perhaps three more years, and we shall then have to return to a volume of smaller size, such as we used to issue. This will be regrettable, and members can postpone that day in three ways. Firstly, by contributing papers which enhance the reputation and indispensability of the *Annals*: the larger part of our income comes from sales to non-members, and these depend upon the standing in the scientific world of the *Annals*. Secondly, by obtaining new members to more than replace our losses by death and resignation; much might be done in this way, if members would make a little effort. Thirdly, by prompt payment of the subscription: in 1936, the arrears were £37. 10*s.* 0*d.*, in 1937, they were £60, in 1938 they were £105. This delay reduces our working balance, and so affects the size of volume we can afford to produce.

J. HENDERSON SMITH
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1938 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated Value of Stock, 1st January 1938		189 19 0	By Sales—Current Volume		628 4 2
To Cambridge University Press		1255 4 10	By Sales—Back Volumes, Parts and Sets		159 12 11
To Copies bought in		15 15 0	By Sales of Reprints		134 18 10
			By Advertisement		1 3 0
			By Estimated Value of Stock, 31st December 1938		150 16 0
			By Balance, carried down		386 3 11
		£1460 18 10			£1460 18 10

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1938 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		386 3 11	By Members' Subscriptions:		
To Printing and Stationery		9 2 5	Arrears	12 10 0	
To Postages and Cheque Stamps		6 18 11	Entrance Fees	8 8 0	
To Honorarium		5 5 0	Current	303 15 0	
To Subscription—Parliamentary Science Committee		10 10 0			324 13 0
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		18 0 11	By Interest on National Savings Certificates and Bank Deposit		25 4 2
To Audit Fee		4 4 0	By Balance, being Excess of Expenditure over Income for the year		90 8 0
		£440 5 2			£440 5 2

BALANCE SHEET, 31 December 1938

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash:		
Cambridge University Press	347 0 11		At Bank on Current Account	82 6 4	
Audit Fee Reserve	4 4 0		At Bank on Deposit Account	1 0 0	
Sundry Expenses	16 19 10				83 6 4
		368 4 9	Debtors for Subscriptions, two years or ess in arrear and considered good		105 0 0
Subscriptions and Entrance Fees, paid in advance		14 12 0	500 National Savings Certificates		806 5 0
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* at estimated value		150 16 0
As Balance Sheet of 31st December 1937	852 18 7				
Less: Balance of Income and Expenditure Account for 1938	90 8 0				
		762 10 7			
		£1145 7 4			£1145 7 4

J. HENDERSON SMITH, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 7 *February* 1939